Sodium/Proton Antiporter Activity is Essential for Virulence of *Yersinia pestis*

Wyatt J. Faulkner[2], Amit Ghosh[1], Yusuke Minato[1], Judith Winogrodzki, Erin Lind[1], Gregory V. Plano[3], Joseph B. Hinnebusch[4], Pavel Dibrov[5], Claudia C. Häse[1,2*]

1 Department of Biomedical Sciences, College of Veterinary Medicine, 2 Department of Microbiology, College of Science, Oregon State University, Corvallis, OR 97331
3 Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, Florida 33101
4 Laboratory of Zoonotic Pathogens, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA.
5 Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

Abstract

We found that a strains of *Yersinia pestis* (KIM5) which lacked the *nhaA* gene was fully attenuated in a plague model. This gene produces a protein of the sodium-proton antiporter family which expel sodium ions from the bacterial cytoplasm in exchange for hydrogen ions, or protons, from the surrounding environment. A *Y. pestis* strain that contained the *nhaA* mutation showed a significant decrease in its ability to survive in both sheep's blood and serum. Decreased growth rates were also observed when the *nhaA* deficient strain was tested in the artificial serum media Opti-MEM® when compared to the wild type strain. A similar growth phenotype was observed when wild type and *nhaA* mutant strains were tested in LB media set to mimic pH and salt conditions of blood. These observations indicate that sodium-proton antiport activity of *Y. pestis* is essential for the survival of the bacterium in certain environments, such as the blood of an infected host. 2-aminopyrimidine was used to inhibit NhaA activity, and when tested in Opti-MEM®, bacterial growth rates decreased. These findings lead us to propose that sodium-proton antiporter inhibition is a novel way of treating bacterial blood-borne diseases.

Background

Yersinia pestis is a pathogenic bacterium that causes the fatal disease bubonic plague. Further development of the disease can lead to septicemia or pneumonic plague. During the middle ages this disease was known as "The Black Death" for the necrosis of tissue that occurred during infection. It was responsible for killing over one quarter of the people living in Europe during that time period. Through medical advances and the discovery of antibiotics, treatment of plague has become relatively safe and successful when combined with proper and quick diagnosis. However, due to its high infectivity and lethality, *Y. pestis* has also recently become considered a potential bioterrorism agent. This particular pathogen is a blood-borne disease and is transmitted to a host via a flea or by an already infected animal. The sodium levels that occur naturally in the blood of animals can cause a fatal shift in the amount of sodium in the cell for *Y. pestis*.*Y. pestis,* as well as other pathogens, protect cells from sodium toxicity by expelling sodium from their cytoplasm. There are two types of sodium pumps, primary and secondary. NADH quinone reductase (NQR) is a primary pump while the secondary pumps are called NhaA and NhaC. The roles of sodium pumps are to not only prevent the intracellular sodium levels to become toxic, but also to generate a gradients so that the sodium motive force (SMF) can continue. Much like the the proton motive force (PMF) the SMF is controlled by the amount of sodium inside and outside of a given cell. It is this role of these secondary sodium pumps that are the focus of this investigation to whether or not sodium/proton antiporters are essential for the virulence of *Y. pestis* in a host.

Conclusions

•The *nhaA* gene is essential for virulence of *Y. pestis,* likely due to the fact that without it, bacterial growth in blood or serum is hindered.

•Death occurs due to the levels of sodium that occur naturally in the blood, 140 mM. Without the NhaA protein the levels become toxic to the cell and it quickly dies.

•Inhibiting the NhaA protein with 2-aminopyrimidine shows a growth defect in the wild type strain in Opti-MEM® similar to that of the *nhaA* deletion.

•We hypothesize that the inability to grow due to the salinity of the blood of a host would allow for the host's immune system to clear the infection before it becomes too overwhelming for the body to deal with.

References

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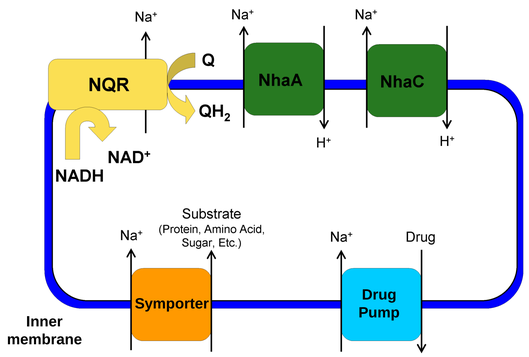
2) Han Y., Zhou, D., *et al*. "Comparative transcriptome analysis of Yersinia pestis in response to hyperosmotic and high-salinity stress". *Research in Microbiology*. 156:3. 2005. 403-415.
3) Häse, C. C., Barquera, B. "Role of sodium bioenergetics in Vibrio cholerae". *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1505:1. 1 2001. 169-178.

A)



B)

Locus	Putative function	
YPO3235 YPO3236 YPO3237 YPO3238 YPO3239 YPO3240	Na^+-translocating NADH:quinone oxidoreductase (NQR) (multisubunit)	Primary Sodium Pumps
YPO0470 YPO0624 YPO2142 YPO3630	Na^+/H^+ antiporter	Secondary Sodium Pumps
YPO0035	sodium/glutamate symporter	Na^+ Dependent Symporters
YPO0251	sodium/proline symporter	
YPO0254	sodium/glutamate symporter	
YPO0584	sodium/dicarboxylate symporter	
YPO0759	sodium/sulfate symporter	
YPO0995	sodium/galactoside symporter	
YPO1008	sodium/sulfate symporter	
YPO1716	sodium/dicarboxylate symporter	
YPO1853	sodium/proline symporter	
YPO2561	sodium/sulfate symporter	
YPO3657	sodium/pantothenate symporter	
YPO3992	sodium/dicarboxylate symporter	
YPO2392	Na^+-dependent multidrug efflux pump	Drug Efflux Pump

Figure 1) The sodium dependent cycle in *Y. pestis* (A) and predicted sodium dependent systems (B).

Genome sequence analyses revealed that *Y. pestits* has two types of sodium efflux pumps, primary sodium pump, NQR, and secondary sodium pumps and multiple sodium dependent transporters.

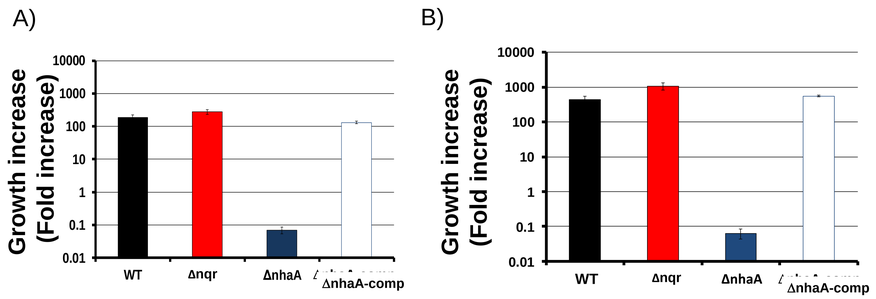


Figure 3) Survival of *Y. pestis* in sheep's blood (A) and sheep's serum (B).

The wild type (WT), *nqr* (Δnqr) and *nhaA* (ΔnhaA) mutants, and the pWSK130nhaA complemented strain (ΔnhaA-comp) were tested in both sheep's blood andsheep's serum to measure growth. After incubation, cultures were diluted and plated so that colony forming units (CFU) couldbe counted to make a quantitative assessment of the survival that occurred. Again a major defect is seen in the *nhaA* mutant, but not in the wild type or the *nqr* mutant, and the defect is corrected upon complementing the *nhaA* mutant strain with the *nhaA*-encoded plasmid.

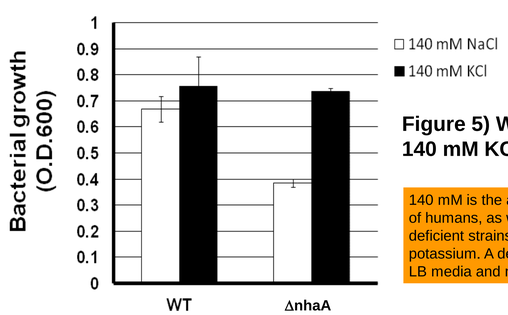


Figure 5) Wild type and *nhaA* mutant growth in 140 mM KCl and NaCl LB media.

140 mM is the average concentration for sodium levels in the blood of humans, as well as most other animals (1). Wild type and *nhaA* deficient strains were tested in LB media containing 140 mM sodium or potassium. A defect was only seen in the *nhaA* mutant in the sodium LB media and not potassium.

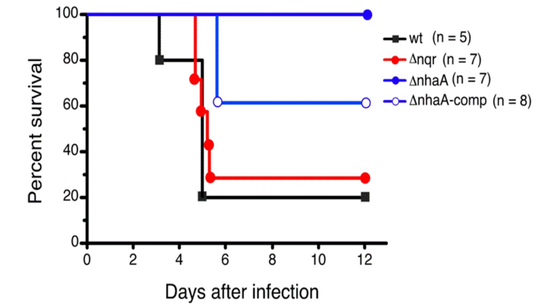


Figure 2) Effects of sodium pump mutations on *Y. pestis* virulence in a murine bubonic plague model.

The *nqr* deletion mutant strain(Δnqr) produced around the same survival rate in mice as did the wild type strain (wt). Whereas the *nhaA* deletion mutant strain (ΔnhaA) showed survival in all of the test subjects. The *nhaA* gene was cloned into the plasmid pWSK130 and electroporated into the Δ*nhaA* strain for complementation purposes. When this strain (ΔnhaA-comp) was te , death still ensued for some of the animals, but survival rates were still higher than those from the *Δnqr* and wild type strains.

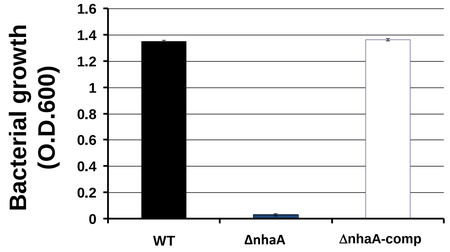


Figure 4) *Y. pestis* strains grown in Opti-MEM® artificial serum media.

The wild type, Δ*nhaA,* and Δ*nhaA*+pWSK130nhaA strains were tested in Opti-MEM®. Almost no growth was observed when *nhaA* was deleted, but when complemented to the pWSK130nhaA plasmid, full growth was seen in the Opti-MEM®.

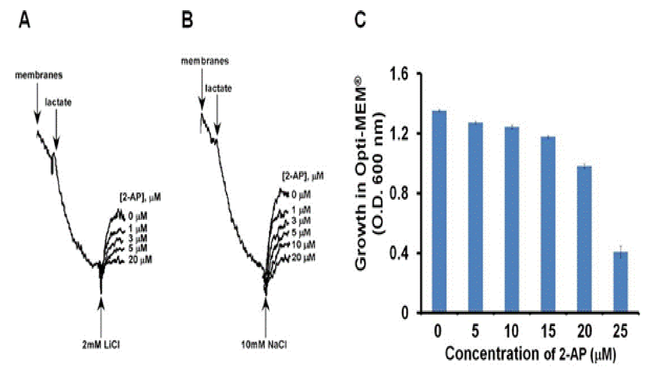
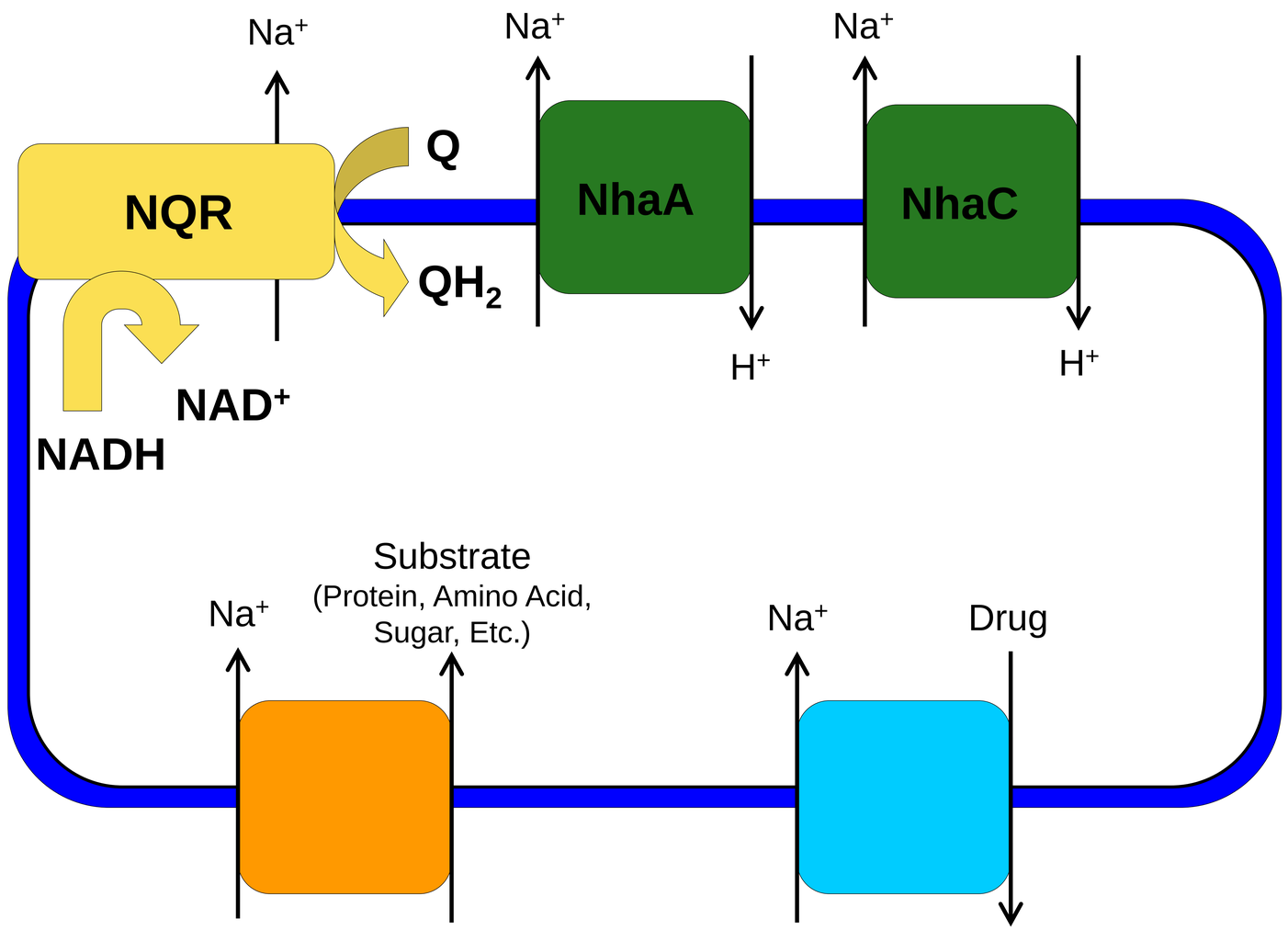


Figure 6) Activity of the NhaA protein of *Y. pestis* in the presence of 2-aminopyrimidine (2-AP).

2-aminopyrimidine (2-AP) is a chemical that inhibits the activity of the NhaA protein. As the amount of 2-AP in solution increased, the activity of the NhaA protein decreased (A, B). Different concentrations of 2-AP were added to Opti-MEM® artificial serum media and inoculated with *Y. pestis*. Increasing concentrations of 2-AP decreased the growth rates of the bacteria.


Na+
Na+
Na+
Q
NQR
NhaA
NhaC
QH2
H+
H+
NAD+
NADH
Substrate
(Protein, Amino Acid,
Sugar, Etc.)
Na+
Na+
Drug