

pH Dependent cAMP Levels Correspond to Acid Resistance in *Escherichia coli*

Shanté Jackson '13 with Joan L. Slonczewski

Kenyon College Summer Science 2010



ABSTRACT

Many pathogenic strains of *Escherichia coli* (*E. coli*) use acid resistance as one of the more important properties of their virulence—the ability to produce disease. It has been predicted that acid resistance is key for *E. coli*'s survival through the acidic environment of the human stomach. Cyclic adenosine monophosphate (cAMP) is a known regulator for many functions in *E. coli*. Among these functions is cAMP's ability, in conjunction with the cAMP receptor protein (CRP), to regulate the expression of acid resistance through the extensive regulatory loop composed of the cross regulation of decarboxylase genes and other protein repressing regulators. In this study, wild type, *cyaA* mutant, and *crp* mutant strains of *E. coli* were placed in both acidic and alkaline (basic) pH environments. From the cultures, cAMP levels were taken in order to see if there was in fact a definite trend between extracellular pH and cytoplasmic cAMP levels. For the mutant strains, it was expected that the lower the pH, the lower the levels of cAMP would be in the bacteria's cytoplasm. This trend also led to the expectation that there would be a higher survival rate at the acidic pH of 5.5 as compared to the alkaline pH of 8.0. The wild type strain was expected to do well regardless of the conditions since it has all the components of the acid resistance regulatory loop. Understanding these regulatory factors would lead to a greater grasp of the behavior of *E. coli* in various environmental conditions.

INTRODUCTION

- Escherichia coli* has an internal pH= 7.4 but is able to survive in acidic conditions—like those found in the human stomach which has a pH= 2.5—(2) due to glutamate decarboxylases which replace a carboxyl group with a proton, consume that proton, in turn producing CO₂ and an ending product catalyzed from glutamate decarboxylase called GABA (Diagram 1. A) (1). The consumption of the proton helps *Escherichia coli* maintain a reasonable internal pH because having more protons present in the cell causes an increased acidic pH.

- Cyclic adenosine monophosphate (cAMP) is derived from adenosine triphosphate (ATP) when it is catalyzed by adenyl cyclase (*cyaA*), a membrane enzyme. Since the *cyaA* enzyme is pH dependent, it corresponds that cAMP levels can be regulated by pH.

- cAMP function is also shown to be dependent on the media in which the bacteria are grown and exposed (treated) in. The importance of the media is noted because it provides the carbon source for the cAMP in order to control the process of cAMP's interactions with CRP and CRP's binding traits to DNA.

MATERIALS AND METHODS

Acid Survival Assay:

- Escherichia coli* was grown overnight in buffered potassium supplemented Luria broth (LBK) pH 8.0 (100mM TAPS) or pH 5.5 (100mM MES). Exposed overnight cultures were diluted 1:200 into 2mL LBK pH 2.0 and incubated at 37°C for 2 hours, further diluted into unbuffered LBK, then plated on plain LBK plates. Untreated overnight cultures were diluted 1:200 into buffered LBK pH 8.0 (100mM TAPS) or pH 5.5 (100mM MES), further diluted into unbuffered LBK, and immediately plated on plain LBK plates. The $\Delta cyaA$ and Δcrp strains were plated on 50 μ g/mL kanamycin LBK plates. Total dilutions ended up at 1:400,000.

cAMP Assay and Protein Assay:

- Escherichia coli* was grown overnight in buffered potassium supplemented Luria broth (LBK) pH 8.0 (100mM TAPS) or pH 5.5 (100mM MES). Overnight cultures were diluted 1:100 (100 μ L of overnight culture into 10mL of buffered LBK) in 125mL baffled flasks and rotated in a water bath at 260rpm, 37°C until they reached an OD₆₀₀ ≈ 0.4 (mid-log phase). Cultured cells were spun down, lysed, and cAMP levels were assessed using an ELISA CatchPoint cAMP Fluorescent Assay (Molecular Devices) and standardized against protein concentration of the same samples using a Pierce BCA Protein Assay (Thermo Scientific).

RESULTS

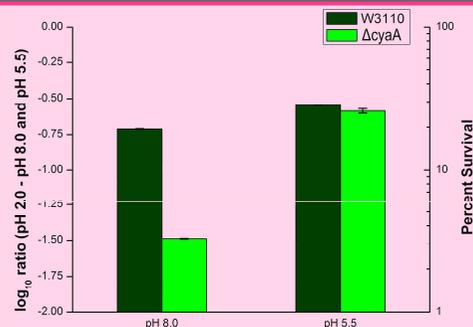


Figure 1. Acid resistance was more present at low pH. The starting pH values were 8.31, 8.11, 5.98, and 5.87 respectively. Error bars represent SEM (n=6).

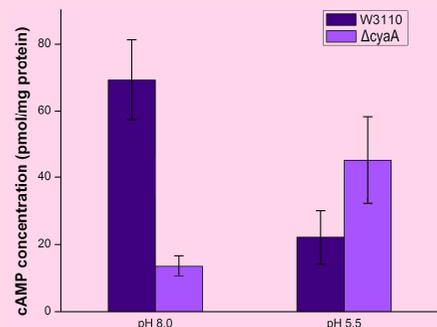


Figure 2. There is a correlation between cytoplasmic cAMP levels and external pH. The trend found was that the higher the pH, the higher the presence of cytoplasmic cAMP. Error bars represent SEM (n=6).

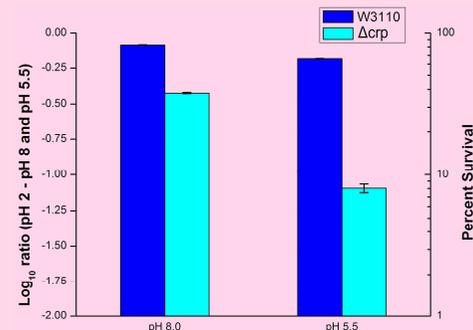


Figure 3. Interestingly, Δcrp displays opposite survival effects in acid compared to $\Delta cyaA$. The starting pH values were 8.27, 7.96, 5.96, and 5.81 respectively. Error bars represent SEM (n=6).

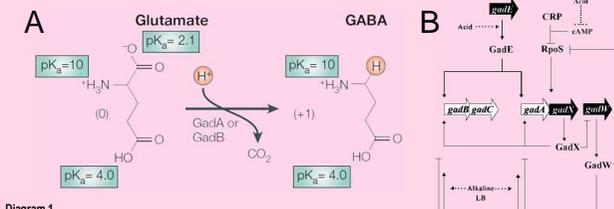


Diagram 1.
A: The process by which glutamate decarboxylase genes regulate internal pH in *Escherichia coli* (1).
B: The relationships between various acid resistance regulatory factors in *Escherichia coli* (3).

CONCLUSION

- Low pH causes low cAMP levels leading to an increase in acid resistance.** In a previous study done by John Foster, it was shown that the lower the external pH, the lower the level of cAMP present in the cytoplasm of the bacterial cell. Since cAMP blocks the production of the RNA polymerase sigma factor (RpoS), as shown in Diagram 1. B, the lower the level of cAMP, the lower the amount of regulation causing the trend seen with resistance in different environmental conditions.

- There is a correlation between cytoplasmic cAMP levels and external pH which, in turn, affects acid resistance in *Escherichia coli*.** As external pH for *Escherichia coli* was manipulated (made acidic or alkaline), the levels of cytoplasmic cAMP were also affected showing that the higher the pH, the higher the cAMP levels as well as visa versa (Fig. 3).

- The Δcrp strain should display the same acid survival trend as the $\Delta cyaA$ strain.** The cAMP receptor protein (*crp*) should block the production of RpoS, suppressing expression of the decarboxylase genes (no acid resistance). The *crp* mutant (Δcrp) would show expression of RpoS, turning on acid resistance. The results we would expect to see is that there would be greater survival at pH 5.5 in acid shock than there would be at pH 8.0 in acid shock. Since cAMP and the *crp* act as a complex, it's unlikely that cAMP is acting alone in activating the decarboxylase genes.

REFERENCES

- Foster, John W. "Escherichia Coli Acid Resistance: Tales of an Amateur Acidophile." *Nature Reviews Microbiology* 2 (2004): 898-907. *Nature Reviews Microbiology*. Web. 15 July 2010. <<http://www.nature.com/nrmicro/journal/v2/n11/pdf/nrmicro1021.pdf>>.
- Jung, Il Lae, and In Gyu Kim. "Polyamines and Glutamate Decarboxylase-based Acid Resistance in *Escherichia coli*." *The Journal of Biological Chemistry* 278th ser. 278:June 20 (2003): 22846-2852. *JBC*. JBC Papers in Press. Web. 15 July 2010. <<http://www.jbc.org/content/278/25/22846.full.pdf+html>>.
- Ma, Zhuo, Hope Richard, and John W. Foster. "PH-Dependent Modulation of Cyclic AMP Levels and GadW-Dependent Repression of RpoS Affect Synthesis of the GadX Regulator and *Escherichia coli* Acid Resistance." *Journal of Bacteriology* 233rd ser. 185 (2003): 6852-859. *JB*. American Society for Microbiology. Web. 15 July 2010. <<http://jfb.asm.org/cgi/reprint/185/23/6852>>.

ACKNOWLEDGEMENTS



I would like to thank Dr. Joan Slonczewski and Ryan Kitko for their guidance throughout all my experiments.

I would also like to thank Kotiba Malek and Kari Deiningner for their willingness to assist me when I needed an extra hand.