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The Binding Characteristics of IclR Transcription Factor

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Abstract
Targeting transcriptional regulators in prokaryotes has proven to be a promising antimicrobial therapy. Regulation of the glyoxylate shunt is directly involved in the metabolic processes and survival of pathogenic bacteria and fungi. The glyoxylate shunt is not found in humans, making it an ideal target. The Isocitrate lyase regulator (IclR) transcription factor found in E. coli is responsible for the expression of the aceBAK operon and the activation of the glyoxylate shunt, which is upregulated under low nutrient stress conditions. Three different IclR binding sites are responsible for the regulation of the glyoxylate shunt and are differentially affected by the binding of glyoxylate and pyruvate to IclR. When pyruvate is present, a tetramer (four similar subunits bound together) is formed which allows IclR to bind to sites one and two upstream of the aceBAK operon, preventing transcription. In contrast, when glyoxylate is present the dimer (two similar subunits bound together) form is preferred and transcription is allowed. Serine 147 of IclR was believed to be significant in the binding of IclR to its DNA target, the aceBAK operon. Using gel shift assays, we determined the binding affinity of the different multimeric (variable number of similar subunits bound together) states of IclR in the presence of its ligands to its DNA targets.

Introduction

Tuberculosis (TB) is currently the most lethal bacterial infectious agent on the planet, killing millions of people per year. The need for new treatments and vaccines is increasing rapidly, and the current rate of development for vaccines and treatments is not high enough to meet the demand (Schranger, Vekemans, Harris, 2018). Tuberculosis strains have become resistant to current antibiotics, largely due to the misuse of drugs prescribed to the patients. In 2016, over 10 million cases of TB were observed. Over 1.6 million people died from TB, and 250,000 of those were due to rifampin resistant and multi-drug resistant strains of TB. HIV positive individuals were at a much higher risk than the general population, and HIV positive individuals alone made up roughly 3.6% of all cases of TB in 2016. Recent projections indicate that if a vaccine with 60% efficacy were to be developed that protected an individual for 10 years, up to 17 million cases of TB could be averted by 2050 (Schranger et al., 2018). The need for a new drug/therapy for TB with high efficacy

cannot be overstated.

M. tuberculosis will activate a pathway called the glyoxylate shunt when in the presence of acetate or fatty acids, and no glucose is available. This is done to prevent carbon loss in the citric acid cycle. The carbon dioxide saved by the glyoxylate shunt is used in gluconeogenesis, which produces pyruvate. As glyoxylate and pyruvate differentially affect the stability of IclR oligomers, one possible explanation for the production of both would be the optimization of the expression and repression of the aceBAK operon. The presence of glyoxylate determines the pathogenicity of *M. tuberculosis* (Lorca G et. al. 2007). If glyoxylate is present *M. tuberculosis* will be pathogenic. The regulation of the aceBAK operon in the glyoxylate shunt appears to be a possible drug/therapy target candidate, as the aceBAK operon is present in *M. tuberculosis* but not humans (Zhou et. al. 2012). This would mean that damage would not occur to a person if a potential therapy disrupted the pathway.

Transcription factors are a type of protein that inhibit or promote the expression of a gene. They do so by binding to a promoter, which is an area where RNA polymerase binds to start transcription. The IclR transcription factor is differentially bound to its promoter depending on the presence of different small effector molecules. When in the presence of glyoxylate, the transcription factor will dimerize (form two subunits) and bind to part of the promoter so that transcription of the aceBAK operon may occur. In the presence of pyruvate, the transcription factor will tetramerize (form four subunits) and bind to the promoter in a way that does not allow transcription of the aceBAK operon to occur (Lorca, G et. al. 2007).

Mutant bacteria may be produced by a process called alanine scanning, which replaces a desired amino acid with an alanine. This may be used to determine the function of different amino acids within the protein, and how the protein interacts with other molecules. Here serine 147 was replaced with an alanine to determine binding affinity.

Experimental Procedure

PCR:

Standard PCR was performed to amplify template DNA. 5 µL Pfx50 PCR buffer, 1.5 µL of both forward and reverse primers (10 µM), 1.5 µL dNTP (10 mM), 1 µL template DNA, 1 µL Pfx50 DNAP, and 40 µL of PCR grade water were added to a tube and amplified using program 900 on the PCR thermal cycler.

Gel electrophoresis:

A 1% agarose gel was used to run the PCR product and a QIAquick gel extraction kit was used to purify the product.

EMSA:

5 ng/µL of 110.5 ng/µL aceBAK promoter DNA was used to prepare 0 nM IclR, 25 nM IclR, 100 nM IclR, and 250 nM IclR (both wild type and S147A) solutions containing 2mM pyruvate and 2 mM glyoxylate. Solutions were incubated at 37º C for 30 minutes, put into a non-denaturing polyacrylamide gel, and ran at 100 volts for one hour and fifteen minutes. The gel was then put into a 1x SYBR green staining solution and shaken for 20 minutes (Figure 1).

Figures

	S147A				WT IclR					
Protein (nM)	250	250	100	25	250	250	250	100	25	0
Pyruvate	-	+	+	+	+	-	+	+	+	+
Glyoxylate	+	-	-	-	-	+	-	-	-	-
Promoter	+	+	+	+	-	+	+	+	+	+

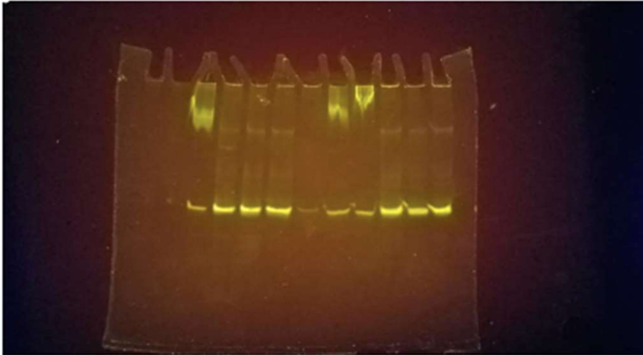

Figure 1. The binding of wild type and S147A IclR to aceBAK promoter DNA. EMSA was performed to show the DNA migration in the presence of wild type IclR transcription factor, and the S147A mutant, with differing amounts of pyruvate and glyoxylate. From right to left: 0nM IclR + Pyruvate (P), 25 nM wild type (WT) + P, 100 nM WT + P, 250 nM WT + P, 250 nM WT + G, 250 nM no DNA + P (with some DNA from the next lane), 25 nM S147A + P, 100 nM S147A + P, 250 nM S147A + P, and 250 nM S147A + G.

Conclusion

The glyoxylate shunt appears to be a promising drug target candidate, as it is not found in humans. The determination of how the IclR transcription factor binds to the aceBAK promoter in the presence of its small effector molecules may provide insight into a novel mechanism which may be exploited for therapeutic purposes. Our S147A mutant demonstrated a lower binding affinity when compared to the wild type (Figure 1). Future work may include looking at the crystal structure and determining other residues that may be important for binding that may be disrupted.

References

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