cAMP Receptor Protein (CRP) Binding Interactions with *WhiB1* from *Mycobacterium tuberculosis*

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BACKGROUND

Mycobacterium tuberculosis is one of the most dangerous human pathogens, claiming ~2 million lives per year. It exhibits extreme antibiotic resistance, which creates a need to better understand the survival mechanisms of *M. tuberculosis in vivo*.

The cAMP receptor protein from *M. tuberculosis* (CRP_{MTB}) is an essential transcription factor that regulates several important genes for host infection and antibiotic resistance in *M. tuberculosis*.

Previous studies have shown a role for the signaling molecule cAMP in enhancing of the highly characterized $CRP_{E.coli}$ binding to specific DNA sequences, which has also been confirmed in CRP_{MTB}.

This study analyzes CRP_{MTB} binding interactions with the *whiB1* gene, which encodes Wb1 proteins that control developmental processes for the pathogen, with and without cAMP through fluorescence anisotropy. The promoter contains two adjacent binding sites which CRP_{MTB} is predicted to bind to with varying affinities.

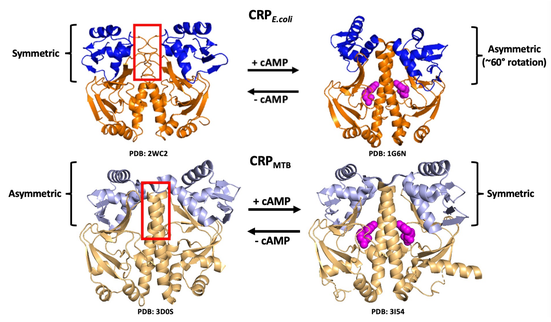


Figure 1. Crystal structures of apo and cAMP-bound $CRP_{E.coli}$ and CRP_{MTB}. Dimerization helix is boxed in red.

MATERIALS AND METHODS

Purification of CRP_{MTB} wildtype protein from *E. coli* strain T7 Express pLysY cells → DNA amplification of the four *WhiB1* promoters of interest by PCR → CRP_{MTB} – *WhiB1* binding interactions monitored by fluorescence anisotropy with and without cAMP

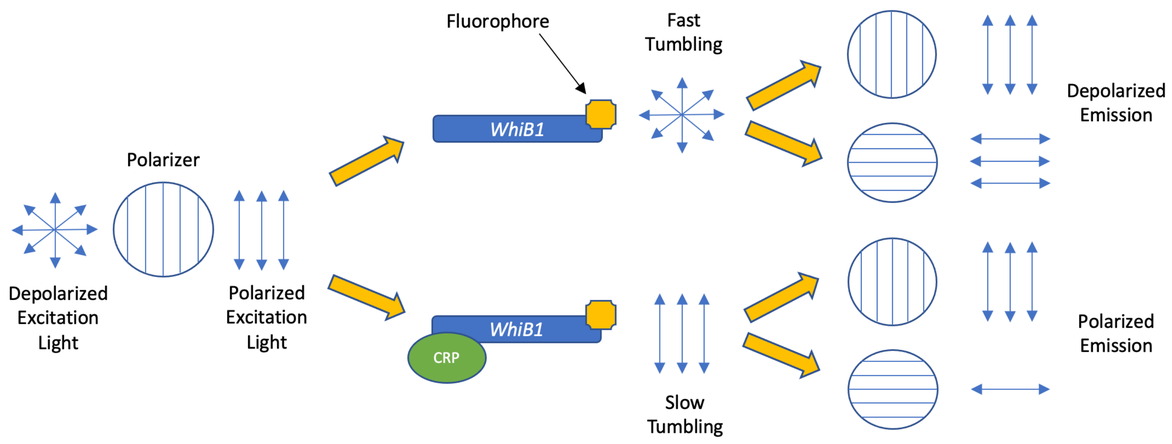


Figure 2. Schematic of the workings of fluorescence anisotropy with generic *WhiB1* construct and CRP.

RESULTS

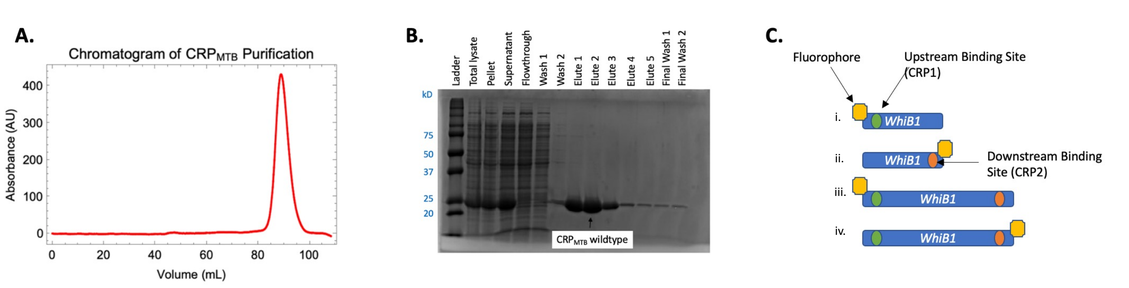


Figure 3. (A) Chromatogram of CRP_{MTB} protein purification. **(B)** SDS-PAGE gel utilized to confirm the presence of CRP_{MTB} wildtype following the size exclusion chromatography at the end of the protein purification process. **(C)** Schematic description of the four *whiB1* constructs (i) *WhiB1 CRP1* (26bp) (ii) *WhiB1 CRP2* (26bp), (iii) *WhiB1 Full CRP1* (48bp), and (iv) *WhiB1 Full CRP2*.

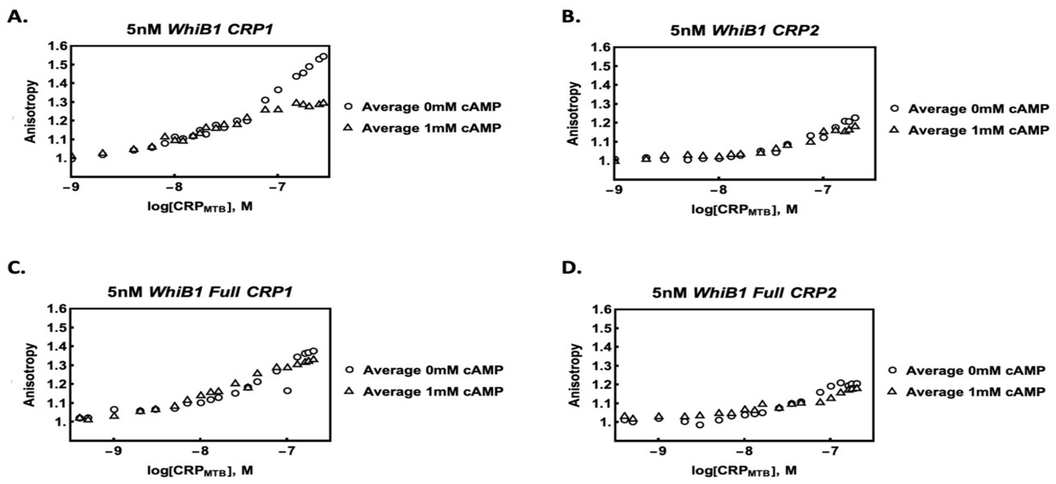
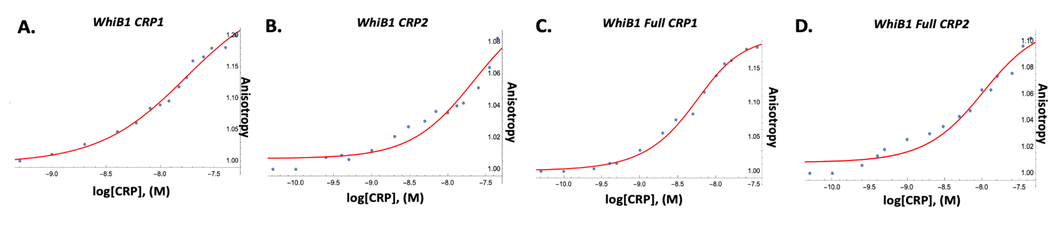


Figure 4. Average anisotropy values for four trials of nonstoichiometric titrations of CRP_{MTB} with and without cAMP for each *whiB1* construct (5nM). Four trials were performed utilizing 0mM cAMP (circles) and four utilizing 1mM cAMP (triangles) for each *whiB1* promoter. **(A)** Average anisotropy values for the *WhiB1 CRP1* (short) 26 bp promoter. **(B)** Average anisotropy values for the *WhiB1 CRP2* (short) 26 bp promoter. **(C)** Average anisotropy values for the *WhiB1 Full CRP1* 48 bp promoter. **(D)** Average anisotropy values for the *WhiB1 Full CRP2* 48 bp promoter.



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Construct	Promoter length	k_{DNA} (cAMP-bound)	
WhiB1 CRP1	26-bp *WhiB1* promoter	7.834	10^{-7} M^{-1}
WhiB1 CRP2	26-bp *WhiB1* promoter	7.721	10^{-7} M^{-1}
WhiB1 Full CRP1	48-bp *WhiB1* promoter	8.547	10^{-7} M^{-1}
WhiB1 Full CRP2	48-bp *WhiB1* promoter	8.116	10^{-7} M^{-1}

Figure 5. Quantification of the functional behavior of CRP_{MTB} when binding with each *whiB1* promoter in the presence of cAMP. Average anisotropy values of CRP_{MTB}-*whiB1* binding with 1mM cAMP are represented by blue circles, while solid red lines represent the mathematical fit created using **Equation 1.** DNA-binding affinities also included (k_{DNA}). **(A)** Fitted data for the *WhiB1 CRP1* promoter. **(B)** Fitted data for the *WhiB1 CRP2* promoter. **(C)** Fitted data for the *WhiB1 Full CRP1* promoter. **(D)** Fitted data for the *WhiB1 Full CRP2* promoter. **(E)** Table of DNA-binding affinity constants of the constructs in the cAMP-bound state.

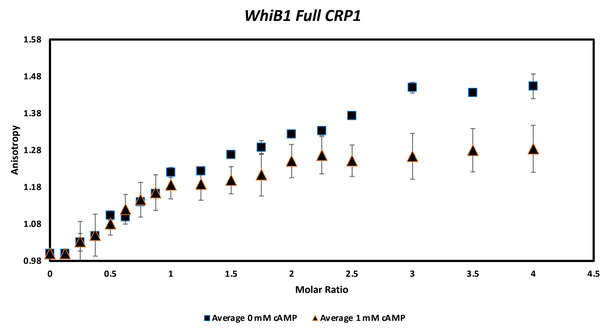

Figure 6. Preliminary stoichiometric binding assay results for the binding between CRP_{MTB} and the *WhiB1 Full CRP1* promoter with and without cAMP. Stoichiometric titration was performed utilizing 200 nM of the *WhiB1 Full CRP1* promoter and up to 800 µM of CRP_{MTB} for two trials with 0mM cAMP (blue squares) and two trials with 1mM cAMP (orange triangles). The anisotropy values from the trails were then averaged and normalized to the first point.

CONCLUSIONS

CRP_{MTB} binds with **higher affinity** to the **upstream** *WhiB1* CRP1 binding site, but the sites are not exhibiting negative cooperativity on one another, as binding still occurs at the downstream *WhiB1* CRP2 site. This study also confirms the previously studied role of cAMP on CRP_{MTB} binding interactions, in that in the presence of cAMP, **high-ordered CRP-DNA oligomers are prevented.**

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