

# Impact of poly-histidine tag on HlyU protein of *Vibrio cholerae*

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## ABSTRACT

ToxR, Fur, and HlyU regulatory systems control the virulence genes in *Vibrio cholerae*. The *V. cholerae* HlyU belongs to the ArsR-SmtB regulatory protein family. The HlyU regulator also controls the production of haemolysin protein HlyA. The likelihood that HlyU protein regulates the expression of virulence factors in addition to HlyA protein was proposed by the abridged colonizing capability of a *hlyU* mutant when compared to a *hlyA* mutant strain. Therefore, the *V. cholerae* HlyU protein and the genes under its regulation are important targets for the development of treatment against *V. cholerae*. In this paper, we showed the implication of using the His<sub>6</sub>-tag for the purification of HlyU protein.

## I. Introduction

Most proteins are not easily amenable to high-throughput analysis because of their varied physicochemical properties. Accordingly, affinity tags have become essential tools for structural and functional proteomics analysis [1]. Although initially developed to ease the detection and purification of recombinant proteins, in recent times it has become clear that affinity tags can have a positive impact on the yield [2-4], solubility [5-8], proper folding [9], and even maintaining structural and functional integrity of their fusion partners [10-12]. Nevertheless, reports show negative effects of affinity tags on the fusion proteins, like change in protein conformation [9], lower yield [10], and alteration in biological activity [12] do exist. In the present study, we found the addition of His<sub>6</sub>-tag to HlyU created a problem during purification. The objective was to purify the *Vibrio cholerae* HlyU protein for structural and biochemical studies.

## II. Observations

Poly-histidine tags are one of the most commonly used affinity tags for the purification of proteins. We found that the presence of a large histidine tag at the N-terminal of the *Vibrio cholerae* transcriptional activator HlyU constrains the protein to exist as a monomer in the solution while removal of the tag results in dimerization of the protein. However, circular dichroism and fluorescence spectroscopic studies did not reveal any significant structural differences between the two forms. The N-terminal poly-histidine tag does not affect the cysteine reactivity of HlyU protein, which was found to be similar in both the monomeric and dimeric forms (His<sub>6</sub>-tagged and tagless HlyU, respectively). Therefore, most probably the N-terminal region is important in forming the dimeric interface of HlyU, and the 34-residue long His<sub>6</sub>-tag was creating some kind of hindrance to the formation of the dimer without significantly altering the overall structure of the protein (Figure 1).

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