Impact of poly-histidine tag on HlyU protein of Vibrio cholera

Rudra P. Saha,*a Manoj K. Singh,a Abinit Saha,a and Arpita Das

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_6-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 His6-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 (His6-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 His6-tag was creating some kind of hindrance to the formation of the dimer without significantly altering the overall structure of the protein (Figure 1).

Department of Biotechnology,
School of Life Science & Biotechnology,
Adamas University, Kolkata 700126, India
**Corresponding author email id: rudrasaha@gmail.com; rudraprasad.saha@adamasuniversity.ac.in*

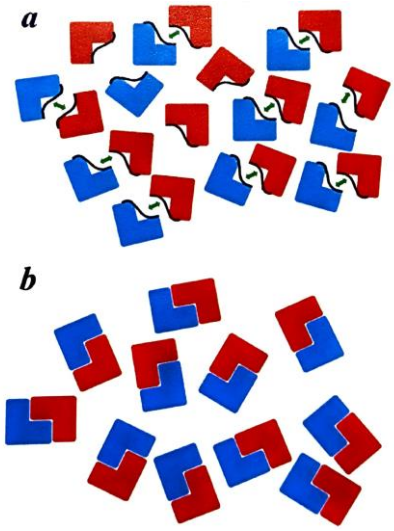

Figure 1. Effect of poly-histidine tag on HlyU protein oligomerization. (a) In presence of a long N-terminal His6-tag the protein exists as a monomer in solution due to the possible steric hindrance (shown as a double-sided green arrow) of the positively charged His6-tag, (b) while after removal of the His6-tag the protein forms a dimeric structure in solution. Two monomers are shown in red and blue colors, respectively.

III. Future Prospects

It has been reported earlier that the presence of the long affinity tags may alter the structure as compared to the native form (Chant et al., 2005), may contribute to conformational heterogeneity allowed by the flexibility of the large tag (Bucher et al., 2002; Smyth et al., 2003), or may present a steric hindrance to ligand or substrate binding and reduce the biological activity of the fusion protein (Fonda et al., 2002). The fact that the addition of large affinity tags to the protein may have undesirable effects on the structure of the protein and thus it is advisable to be cautious when using His-tagged proteins directly in research works (Saha et al., 2006; Saha and Chakrabarti, 2006).

Acknowledgment

The authors would like to thank Bose Institute, Kolkata, and Adamas University, Kolkata, for providing laboratory supports.

References

[1] Sun, Q. M., Chen, L. L., Cao, L., Fang, L., Chen, C., & Hua, Z. C. (2005). An improved strategy for high-level production of human vasostatin120-180. Biotechnology Progress, 21(4), 1048-1052.

[2] Waugh, D. S. (2005). Making the most of affinity tags. Trends in Biotechnology, 23(6), 316-320.

[3] Rajan, S. S., Lackland, H., Stein, S., & Denhardt, D. T. (1998). Presence of an N-terminal polyhistidine tag facilitates stable expression of an otherwise unstable N-terminal domain of mouse tissue inhibitor of metalloproteinase-1 in Escherichia coli. Protein Expression and Purification, 13(1), 67-72.

[4] Chen, H., Xu, Z., Xu, N., & Cen, P. (2005). Efficient production of a soluble fusion protein containing human beta-defensin-2 in E. coli cell-free system. Journal of Biotechnology, 115(3), 307-315.

[5] Dyson, M. R., Shadbolt, S. P., Vincent, K. J., Perera, R. L., & McCafferty, J. (2004). Production of soluble mammalian proteins in Escherichia coli: identification of protein features that correlate with successful expression. BMC Biotechnology, 4, 32.

[6] Hammarström, M., Hellgren, N., van Den Berg, S., Berglund, H., & Härd, T. (2002). Rapid screening for improved solubility of small human proteins produced as fusion proteins in Escherichia coli. Protein Science, 11(2), 313-321.

[7] Nallamsetty, S., & Waugh, D. S. (2006). Solubility-enhancing proteins MBP and NusA play a passive role in the folding of their fusion partners. Protein Expression and Purification, 45(1), 175-182.

[8] Kou, G., Shi, S., Wang, H., Tan, M., Xue, J., Zhang, D., Hou, S., Qian, W., Wang, S., Dai, J., Li, B., & Guo, Y. (2007). Preparation and characterization of recombinant protein ScFv(CD11c)-TRP2 for tumor therapy from inclusion bodies in Escherichia coli. Protein Expression and Purification, 52(1), 131-138.

[9] Tang, W., Sun, Z. Y., Pannell, R., Gurewich, V., & Liu, J. N. (1997). An efficient system for production of recombinant urokinase-type plasminogen activator. Protein Expression and Purification, 11(3), 279-283.

[10] Mayer, A., Sharma, S. K., Tolner, B., Minton, N. P., Purdy, D., Amlot, P., Tharakan, G., Begent, R. H., & Chester, K. A. (2004). Modifying an immunogenic epitope on a therapeutic protein: a step towards an improved system for antibody-directed enzyme prodrug therapy (ADEPT). British Journal of Cancer, 90(12), 2402-2410.

[11] Wang, X., Campoli, M., Ko, E., Luo, W., & Ferrone, S. (2004). Enhancement of scFv fragment reactivity with target antigens in binding assays following mixing with anti-tag monoclonal antibodies. Journal of Immunological Methods, 294(1-2), 23-35.

[12] Chant, A., Kraemer-Pecore, C. M., Watkin, R., & Kneale, G. G. (2005). Attachment of a histidine tag to the minimal zinc finger protein of the Aspergillus nidulans gene regulatory protein AreA causes a conformational change at the DNA-binding site. Protein Expression and Purification, 39(2), 152-159.