Investigations on *Vibrio cholerae* regulatory protein ToxR

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The cholera causative *Vibrio cholerae* can adapt rapidly to changing environments via sensory proteins like inner membran regulator ToxR, transducing signals from its periplasmic sensory domain to its cytoplasmic effector domain. ToxR thus activates, co-activates or represses numerous genes in *V. cholerae*, among them also virulence associated genes. Previously studies suggested that inner membrane protein ToxS plays a crucial role in the activity of ToxR.

The NMR structure of the periplasmic domain of ToxR (ToxRp) reveals the formation of a four stranded β sheet stacked against a long α-helix [1]. C236, in the middle of the helix forms a disulphide bond with C293 at the C-terminal end. NMR dynamic studies showed that under reducing conditions ToxRp adapts two conformations: one resembling the oxidized form, and a second one revealing strong dynamics proposing an unstructured form. The long C-terminal stretch, including C293, seems to be unstructured and highly flexible under reducing conditions, thereby suggesting an explanation for the increased proteolytic sensitivity of reduced ToxR (ToxRp-red) that was previously reported in *V. cholerae* [2,3].

By using a combination of NMR, SEC-MALS, and Fluorescence Anisotropy we could identify the formation of a strong heterodimer of the periplasmic domains of inner membrane proteins ToxR and ToxS (ToxRSp) independent on the redox state of ToxRp. Our results reveal that ToxRp binds ToxSp in a 1:1 fashion with a dissociation constant of 11.6nM. Additionally, by monitoring the proteolytic cleavage of ToxRp with NMR we provide a direct evidence of ToxS protective function.

The versatile functions of ToxR propose separate control mechanisms, in order to regulate the activity of ToxR as direct activator, co-activator or repressor. We propose that ToxR activity is mainly controlled by its stability. The reduction of ToxRp cysteines represent one possibility to decrease ToxR stability. This regulation is controlled by periplasmic oxidoreductases DsbA and DsbC *in-vivo* [2-4]. The interaction with ToxS represents another possibility to increase ToxR stability by directly protecting ToxRp from proteases DegPS [2,5]. Binding of ToxS seems to be independent from ToxRp cysteines. Previous work has shown that *V. cholerae* alkalinizes its surrounding in the late stationary phase, which decreases the interaction between ToxRS and subsequently leads to a loss of function of ToxR due to proteolysis [6,7]. Furthermore, our experiments show that binding of ToxSp does not trigger dimerization of ToxRp via disulphide bonds under the applied conditions. Therefore, our data also support the theory that dimerization of ToxR, in order to induce transcription, is activated by the presence of DNA [3,7].

[1] Gubensäk, N., Wagner, G.E., Schrank, E., et al. (2021). Mol Microbiol. 115, 1277– 1291.

[2] Lembke, M., Höfler, T., Walter, A., et al. (2020). Mol Microbiol. 114, 262-278.

[3] Lembke, M., Pennetzdorfer, N., Tutz S., et al. (2018) Mol Microbiol. 110, 796–810.

[4] Fengler, V. H. I., Boritsch, E. C., Tutz, S., et al. (2012): PloS one 7, e47756.

[5] Pennetzdorfer, N., Lembke M., Pressler, K., et al. (2019). Frontiers in cellular and infection microbiology 9, p. 214.

[6] Almagro-Moreno, S., Kim, T. K., Skorupski, K., et al. (2015a): PLoS genetics 11, e1005145.

[7] Midgett, C. R., Almagro-Moreno, S., Pellegrini, M., et al. (2017): Mol Microbiol. 105, 258–272.

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