S2-2

New Roles of Glucose-Specific Enzyme IIA of the *Vibrio vulnificus* Phosphotransferase System

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Abstract

In a previous report, we showed that enzyme IIA^{Glc} ($EIIA^{Glc}$) of *Escherichia coli* phosphotransferase system (PTS) interacts with and regulates activity of FrsA (fermentation/respiration switch protein). A BLAST search revealed that orthologs of FrsA exist only in some Gram-negative bacteria such as *E. coli, Salmonella typhimurium, Shigella flexneri, Yersinia pestis, Vibrio cholerae, Vibrio vulnificus, Vibrio parahemeolyticus*, and *Photorhabdus luminescens* and all of these species are facultative anaerobes belonging to the γ-proteobacterial group, and most of them are highly pathogenic. Ligand-fishing experiments using $EIIA^{Glc}$ of *Vibrio vulnificus* ($vEIIA^{Glc}$) as bait revealed that $vEIIA^{Glc}$ also interacts with vFrsA in a phosphorylation state-dependent manner. The *frsA* mutant of *Vibrio vulnificus* showed remarkably reduced cytotoxicity to HeLa cells and reduced lethality to mice compared to wild type. Comparison of extracellular proteomes between the mutant and wild type indicated that hemolysin was not produced in the *frsA* mutant. Characterization of another protein interacting with $vEIIA^{Glc}$ will be discussed.

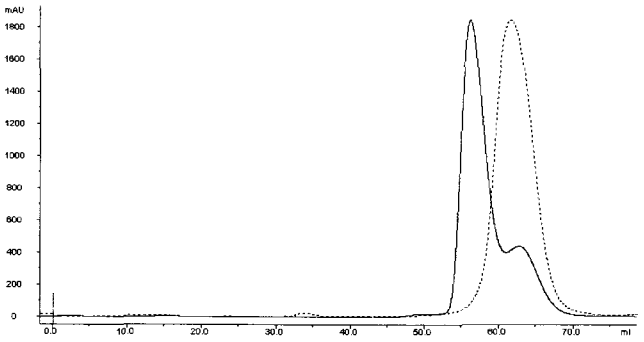
Rationale and Methods

While the PTS has never been studied in *V. vulnificus*, we postulated that the PTS might be implicated in the pathogenicity of *V. vulnificus* by sensing host glucose level, based on the fact that hosts suffering from diabetes are more susceptible to *V. vulnificus* infection and that the PTS regulates many physiological processes by sensing the glucose level in the environment. This perspective stimulated us to embark on searching for new regulatory roles of the PTS, possibly its involvement in the pathogenicity, of *V. vulnificus*. Because all of the regulatory roles of the PTS thus far reported are mediated through the direct interaction of the PTS proteins with their target proteins and IIA^{Glc} plays the most important regulatory roles of the PTS, we set out to find out a protein(s) interacting with and thus being regulated by IIA^{Glc}. To search for a protein(s) interacting with IIA^{Glc}, the crude extract

prepared from *V. vulnificus* was mixed with IIA^{Glc} or 6His-tagged form of IIA^{Glc} (His-IIA^{Glc}) and subjected to a pull-down assay using the BD TALON™ metal affinity resin. Through several independent trials, we could find proteins differentially eluted between columns.

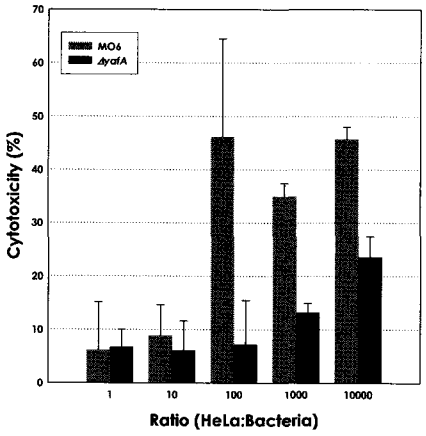
Results

1. Specific interaction between the dephospho-form of $vEIIA^{Glc}$ and vFrsA.



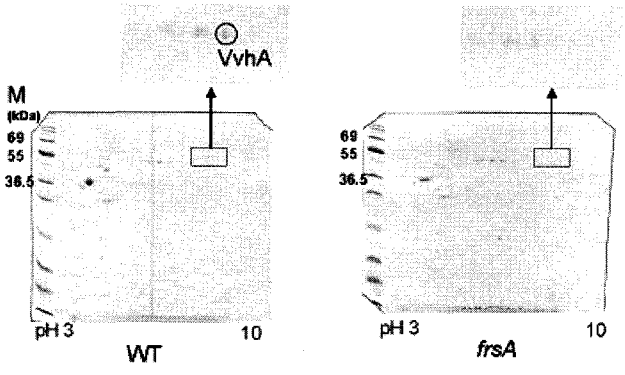
Comparison of elution profiles through a gel filtration column of vFrsA and its complex with $vEIIA^{Glc}$.

2. The *frsA* mutant shows remarkably reduced cytotoxicity to HeLa cells and reduced lethality to mice.

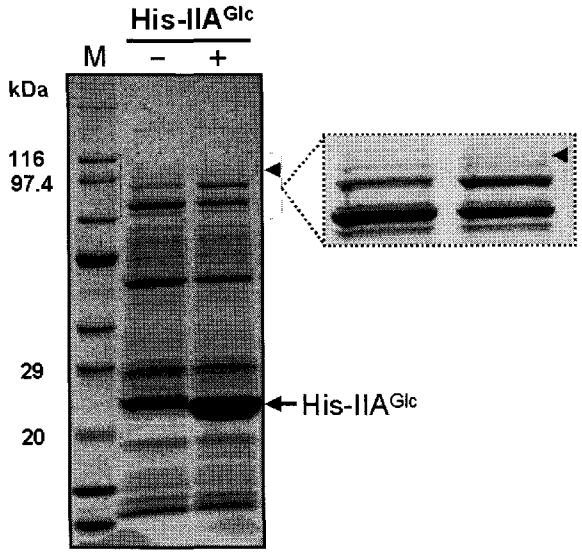


	LD50 (CFU/ml)
Wild type	1.8×10^3
Δ*frsA*	1.6×10^4

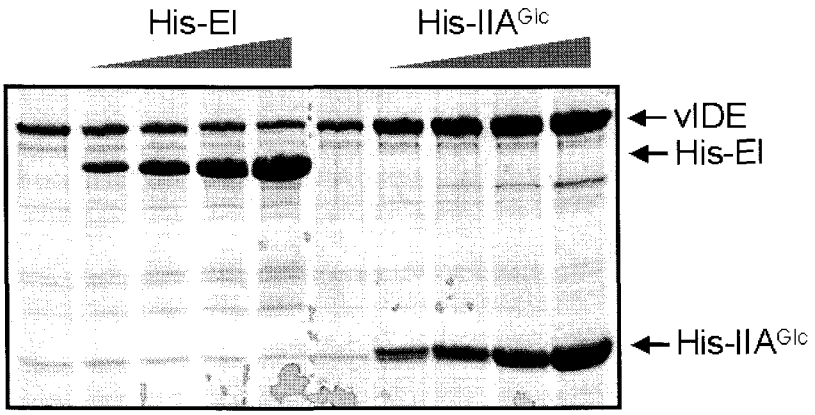
3. Comparison of extracellular proteomes between the mutant and wild type shows that hemolysin is not produced in the *frsA* mutant.



4. Fishing a protein of unknown function interacting with vEIIA.



5. Specific interaction of a 100kDa protein with vEIIA.



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