[SIII-1]

Role of CpdA in Intracellular Concentration of cAMP and Stress-response of *Vibrio vulnificus*

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The fatal septicemia-causing pathogenic bacterium, *Vibrio vulnificus* is believed to transit two distinct niches, a host to proliferate and elicit virulence (human) and an environmental reservoir to survive and translocate (seawater, sediments or seafoods). Therefore, this pathogenic bacterium has evolved to develop several mechanisms to sense the fluctuations in its surrounding conditions and to express the necessary defense elements which allow it to adapt and survive under the stress conditions, for examples, oxidative stress, osmotic shock, acidic stress, and starvation.

Cyclic 3',5'-adenosine monophosphate (cAMP) is considered as an important cellular signal in many bacteria. It has shown to be involved in many processes related to catabolisms; one of the well-known examples is the induction of *lac* operon expression by cAMP-CRP complex. In addition to catabolite repression cAMP has been reported to regulate diverse functions, e.g, pH-regulated gene expression, flagellum synthesis, enterotoxin production, production of outer-membrane proteins, iron uptake, filamentation, synthesis of glycogen and ubiquinone, and etc.

Thus the intracellular concentration of cAMP plays a critical role in global regulatory network. Concentration of intracellular cAMP in bacterial cytoplasm is finely and timely modulated by differential degrees of synthesis by adenylate cyclase (Cya), excretion of cAMP, and degradation into 5'-adenosine monophosphate (AMP) by 3',5'-AMP phosphodiesterase (CpdA). In contract to extensive studies on synthesis and excretion of cAMP, little is known about the enzymatic hydrolysis of cAMP. The genes coding for the cytoplasmic CpdAs have been isolated from *E. coli* and *Haemophilus influenza*. It has been shown that these *cpdA* gene products are involved in reduction of the cAMP levels.

The orf encoding CpdA was cloned from the chromosomal DNA of a human-pathogenic *Vibrio vulnificus* ATCC29307. The deduced sequence of 274-amino acid residues revealed 50.5% identity to the homolog in *E. coli*, CpdA (or Icc). The promoter region of *cpdA*, which has been determined by a primer extension experiment shows the presence of the putative cAMP-CRP binding site at the upstream of –35 region. When cells entered stationary phase in 0.05% yeast extract-treated artificial seawater containing 0.05% glucose as a sole carbon source, dramatic decrease in the expression of the *cpdA* gene, as determined by both Northern blot and *cpdA*'::*luxAB* fusion analysis, occurred with concomitant increase of cAMP concentration within *V.*

vulnificus cell. The decrease in the expression of *cpdA* seems to increase the intracellular concentration of cAMP at the onset of starvation or upon exposure to other stresses.

To investigate a functional role of *cpdA* in *V. vulnificus*, a *cpdA*-null *V. vulnificus* mutant strain was constructed by exchanging the intact *cpdA* allele on the chromosomal DNA with the internally deleted Δ*cpdA*. The resultant strain, HY101, which is deficient in ability to degrade cAMP, surprisingly, showed no accumulation of cAMP along the whole growth periods. This mutant with an elongated cellular morphology (observed under the scanning electron microscope) showed decreased survivalship under some stress conditions. Interestingly, the same phenotypes have been found in the *V. vulnificus* cells containing high copy number of the *cpdA* gene. These results suggest that the adequate amount of cAMP resulted from the appropriate activity of CpdA upon the exposure to the various stress conditions may be required for a better survival.

The increased susceptibility to starvation has been also observed in σ^S-defective (Δ*rpoS*) *V. vulnificus*. Therefore, the relationship between intracellular level of cAMP and synthesis of σ^S was further investigated. The expression of *cpdA* (determined by *cpdA'::luxAB* fusion) is partially dependent upon the presence of σ^S as well as the synthesis of σ^S is depressed in the absence of CpdA activity. These results strongly suggest a possibility that the stress-survival pathways operated by cAMP and σ^S may be overlapped or hierarchically coordinated.

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