Available online at www.sciencedirect.com
SCIENCE DIRECT®

ELSEVIER

Biochemical and Biophysical Research Communications 331 (2005) 1365–1373

BBRC

www.elsevier.com/locate/ybbrc

In vivo induced *clpB1* gene of *Vibrio cholerae* is involved in different stress responses and affects in vivo cholera toxin production

Sanjay Nag, Soumita Das [1], Keya Chaudhuri *

Human Genetics and Genomics Group, Indian Institute of Chemical Biology, Jadavpur, Calcutta 700 032, India

Received 30 March 2005
Available online 20 April 2005

Abstract

Previously in global transcription profile approach one of the cosmid clones of *Vibrio cholerae* containing the genes *pnuC*, *icmF*, and a fragment of *clpB2* showed higher expression in *V. cholerae* grown inside rabbit intestine. In the present report, both the stress responsive *clpB* genes of *V. cholerae* O395 were cloned, *clpB1* from chromosome I and *clpB2* present in chromosome II. From the Northern blot hybridization it was observed that the level of transcription of *clpB2* was very low which could be due to the weak promoter strength of *clpB2* as predicted in silico. The deduced amino acid sequence showed that *clpB1* possesses features typical of the ClpB ATPase family of stress response proteins. The *clpB1* gene showed about three times higher expression under in vivo condition than in vitro. Increased expression of *clpB1* gene was also observed at high temperature, high salt, and in the condition mimicking human intestine viz., 37 °C, pH 8.5, 300 mM NaCl, which is known to be the repressive condition for ToxR, the global transcriptional regulator of virulence in *V. cholerae*. The *clpB1* insertion mutant showed increased sensitivity towards high temperature, oxidative stress, and acid pH. ClpB1 also conferred thermotolerance to *V. cholerae*. These effects could be reversed by complementation. Although *clpB1* appeared not to be under the control of virulence regulatory cascade of *V. cholerae*, the CT production was reduced in *clpB1* mutant when tested in vivo in an infant mice model.
© 2005 Elsevier Inc. All rights reserved.

Keywords: *clpB1*; *Vibrio cholerae*; Stress response; Virulence; CT production; In vivo

Vibrio cholerae, the facultative anaerobic gram-negative γ-proteobacteria, is the etiological agent of the severe diarrheal disease cholera. This unique bacterial pathogen can exist as free-living environmental organism within an aquatic natural reservoir as well as a human intestinal pathogen. The organism enters the host through an oral route of infection, transits the gastric acid barrier of the stomach, and colonizes within the small intestine. Once there, the organism secretes cholera toxin and the life-threatening diarrhea is largely due to the action of CT on the epithelium of the small intestine. Besides CT, additional factors like toxin coregulated pili (TCP), other potential toxins, accessory colonization factors, outer membrane proteins, hemolysins, and hemagglutinins contribute to the pathogenesis of this disease [1]. In *V. cholerae*, the expression of a subset of virulence factors is coordinately controlled by a regulatory cascade in which ToxR and TcpP, the two inner membrane DNA binding proteins, regulate the expression of ToxT, another transcriptional activator that directly regulates the expression of several virulence genes including those coding for CT and TcpA, the

* Corresponding author. Fax: +91 33 2473 5197, +91 33 2473 0284.
E-mail addresses: keyachaudhuri@yahoo.com, kchaudhuri@iicb.res.in (K. Chaudhuri).
[1] Present Address: Department of Pediatrics, UTMB, Galveston, USA.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.04.052

major subunit of TCP [1,2]. A number of parameters like temperature, pH, osmolarity, amino acids, and bile are known to modulate the expression of ToxR regulon, and these environmental signals exert their effects at different levels of the regulatory cascade [3].

Vibrio cholerae, in its transition from the natural environment to the host intestine, is subjected to a plethora of stresses such as fluctuations of temperature, pH, osmolarity, oxygen tension, and nutritional ability [4]. These offer selective pressure to the bacterium, eliciting various adaptive responses for its survival. To identify the genes that are expressed following infection to the host, several approaches like in vivo expression technology [5], signature tagged mutagenesis [6], RNA arbitrarily primed PCR fingerprinting [7], and global transcription profiling [8] have already been used. Comparison of global transcription profile of *V. cholerae* grown in vitro and in rabbit intestine revealed that in addition to the virulence genes *ctxAB*, *toxR*, *toxT*, and *tcpA* that are induced under in vivo condition, one of the in vivo induced cosmids after dissection, subcloning, and sequencing showed homology with the *clpB-2* gene of *V. cholerae* Eltor N16961 [9]. ClpB2 belongs to ClpATPase (also referred to as Hsp100) family of stress response proteins and is involved in protein degradation and disaggregation in both prokaryotic and eukaryotic cells thereby protecting the cells from damage under stress condition [10].

The Clp (caseinolytic protease) system was first identified as a heat shock inducible, multicomponent, ATP-dependent homohexameric protease complex able to hydrolyze casein. Clp family can be divided into two basic groups, with proteins in the first (ClpA-E) having two distinct ATP binding domains (ATP-1 and ATP-2) while proteins in the second (ClpX and ClpY) possess only one such domain [10]. The main Hsp of this chaperone family is ClpB which is a universal protein found in almost all organisms including gram positive and gram negative bacteria, archaebacteria like *Methanosarcina acetivorans*, plants like *Arabidopsis thaliana*, protozoa like *Trypanosoma brucei*, parasites like *Leishmania major*, *Leishmania donovani*, and also in *Drosophila melanogaster* and *Saccharomyces cerevisiae* [11]. ClpB is very much unique and most organisms produce at least two different types. Separate nuclear *clpB* genes in eukaryotes encode mitochondrial (78 kDa [12]) and cytosolic (100–110 kDa [13]) proteins, while plants have another ClpB isomer localized in chloroplasts [14]. In contrast, a single gene in eubacteria encodes two differently sized proteins (79 and 93 kDa) via dual translational initiation sites within the *clpB* transcript [15].

Mechanistically ClpB functions to dissolve inactive protein aggregates that accumulate at high temperature [16]. In both bacteria and eukaryotes, ClpB cooperates with the DnaK-DnaJ-GrpE proteins in bi-chaperone network in vitro to prevent and revert protein aggregates during heat shock [17] which represents an important contribution to the understanding of the unique role of ClpB in thermotolerance and stress recovery. ClpB is unique among the Hsp100/Clp proteins since it does not associate with a proteolytic partner. Recently, it was reported that the signature motif that is conserved in all other Clp interacting proteins like ClpA is missing in ClpB and thereby explains why ClpB acts independently of peptidases [18]. Instead, ClpB mediates the resolubilization of aggregated proteins in cooperation with the DnaK chaperone system, but the mechanism of disaggregation reaction and the basis of ClpB/DnaK cooperation are still not understood. In the complete genome sequence of *V. cholerae N16961*, five *clp* genes *clpA*, *clpX*, *clpP*, *clpB1*, and *clpB2* are present but none of them have so far been experimentally characterized.

The present study is the first report of functional characterization of *clpB1* of *V. cholerae*. The *clpB1* gene was found to be upregulated in vivo inside rabbit intestine and in vitro under the condition of 37 °C, pH 8.5, and 300 mM NaCl. Insertion mutant in *clpB1* gene showed that ClpB1 in *V. cholerae* is involved in protection to heat, oxidative, and acid stresses. Moreover, ClpB is involved in thermotolerance in this organism. Although the virulence cascade of *V. cholerae* does not regulate *clpB1*, the CT production in vivo is reduced in the *clpB1* mutant.

Materials and methods

Bacterial strains and plasmids. The *V. cholerae* O395 and *Escherichia coli* DH5α strains used in this study were maintained at −70 °C in Luria–Bertani (LB) medium containing 20%(v/v) glycerol. *E. coli* cells were grown in LB medium and *V. cholerae* cells in nutrient broth or LB medium. Ampicillin and kanamycin for *E. coli* were used at 100 and 50 μg/ml, respectively. Full genes were cloned in pT-Adv vector (Clontech, USA) and subcloning was done in pBluescriptKS+. For successful complementation, the vector pSKW130 was used which was a generous gift from Prof. Sydney Khusner, University of Georgia, Athens, Greece. The plasmids were maintained and amplified in *E. coli* DH5α and XL1 Blue strains.

DNA preparation and manipulation. Genomic DNA from *V. cholerae* was prepared from proteinaseK digested and cetyl-trimethyl-ammonium bromide precipitated cell lysates by using standard procedures [19]. Plasmid DNA was prepared by the alkaline lysis method [20]. Restriction enzymes and DNA modifying enzymes were purchased from New England Biolabs (Beverly, Massachusetts, USA) and used according to the manufacturer's recommendations.

PCR. For gene specific PCR, the reaction was carried out in 20 μl reaction volume containing 1× PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl], 10% DMSO, 0.2 mM of each dNTP, 5–10 pmol of each primer, optimal concentration of $MgCl_2$ (1.5–2 mM), and 50–100 ng of O395 genomic DNA. After heating at 95 °C for 5 min, the mixtures were cooled to annealing temperature and then 0.5 U *Taq* DNA polymerase (Invitrogen, Life Technologies, USA) was added. The primers were designed through PRIMER3 program at http://www.genome.wi.mit.edu. The 2896 bp amplified product of *clpB1* was obtained using the primers 5′ TAAGCCTTCATCCATACCCA 3′ and

Download English Version:

https://daneshyari.com/en/article/10770482

Download Persian Version:

https://daneshyari.com/article/10770482

Daneshyari.com