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Functional characterization of the *Helicobacter pylori* chaperone protein HP0795

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ABSTRACT

Trigger factor (TF) is one of the multiple bacterial chaperone proteins interacting with nascent peptides and facilitating their folding in bacteria. While TF is well-characterized in *E. coli*, *HP0795*, a TF-like homologue gene identified earlier in the pathogenic *Helicobacter pylori* (*H. pylori*), has not been studied biochemically to date. To characterize its function as a chaperone, we performed 3D-modeling, cross-linking and *in vitro* enzyme assays to HP0795 *in vitro*. Our results show that HP0795 possesses peptidyl prolyl *cis-trans* isomerase activity and exhibits a dimeric structure in solution. In addition, stable expression of HP0795 in a series of well-characterized *E. coli* chaperone-deficient strains rescued the growth defects in these mutants. Furthermore, we showed that the presence of HP0795 greatly reduced protein aggregation caused by deficiencies of chaperones in these strains. In contrast to other chaperone genes in *H. pylori*, gene expression of *HP0795* displays little induction under acidic pH conditions. Together, our results suggest that HP0795 is a constitutively expressed TF-like protein of the prokaryotic chaperone family that may not play a major role in acid response. Given the pathogenic properties of *H. pylori*, our insights might provide new avenues for potential future medical intervention for *H. pylori*-related conditions.

1. Introduction

Chaperones are a family of proteins presented in both prokaryotic and eukaryotic cells, where they fulfill essential functions in nascent peptides folding, in preventing misfolding or aggregation of proteins and in maintaining protein homeostasis. Trigger factor (TF) is an archetypical *E. coli* chaperone, both in terms of its structure as well as its function. In the prokaryotic protein folding pathway, TF is the first chaperone in the pathway, where it interacts co-translationally with newly synthesized polypeptides emerging from the bacterial ribosome. TF maintains the nascent polypeptide in a non-aggregated state until completion of folding. In addition, TF promotes protein folding and participates in both the post-translational assembly of large protein complexes as well as in ribosome biogenesis (Merz et al., 2008; Hartl and Hayer-Hartl, 2009; Martinez-Hackert and Hendrickson, 2009; Hoffmann et al., 2010). *E. coli* TF, with ~50 kDa molecular weight, is composed of three distinct domains: the N-terminal ribosome-binding domain, the C-terminal chaperone domain and the peptidyl-prolyl *cis-trans* isomerase (PPIase) domain in the centre (Kramer et al., 2004b; Merz et al., 2006).

TF exhibits partial functional overlapping with DnaK, which is a central organizer of the chaperone network involved in proper protein folding in *E. coli*. *In vivo,* DnaK was shown to partially compensate TF knockout, as observed by the lack of defects in either growth or detectable protein folding in TF-deficient *E. coli* strains (Deuerling et al., 1999; Teter et al., 1999). In addition to their overlapping functions, TF possesses specific traits in the absent of DnaK. For instance, TF is essential for the biogenesis of outer membrane proteins (Calloni et al., 2012). Importantly, upon double-knockout of both DnaK and TF, *E. coli* growth is suppressed at most physiological temperatures, and was only viable in a narrow temperature range. Importantly, within this range protein aggregates were observed to accumulate (Deuerling et al., 1999; Teter et al., 1999; Genevaux et al., 2004). Together, these findings show that *in vivo*, the cooperation of TF and DnaK is necessary for maintaining proteostasis *in vivo*.

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Helicobacter pylori (*H. pylori*) is the most important pathological bacterium to exclusively colonize the human stomach. Chronic infection with *H. pylori* is the pathological basis of peptic ulcer, stomach carcinoma and B-cell mucosa-associated lymphoid tissue lymphoma (Suerbaum and Michetti, 2002; Kusters et al., 2006; Cover and Blaser, 2009). For successful colonization in the stomach, *H. pylori* has developed a acid-adaptive mechanism, providing it with the ability to survive in the human stomach characterized by a large pH gradient across the organ (Wen et al., 2003; Kusters et al., 2006; Pflock et al., 2006). While a number of cell-intrinsic virulence factors, such as cagA and vacA, have been identified to be involved in virulence and pathogenicity of *H. pylori* (detailed in reviews: (da Costa et al., 2015; Mentis et al., 2015)). However, little research has focused on the field of chaperones involved in the biogenesis of virulence factors in *H. pylori*.

Sequencing of the *H. pylori* 26695 genome has identified 1587 open reading frames (ORFs) (Tomb et al., 1997). Compared with ~4290 ORFs in *E. coli*, the *H. pylori* genome is of relatively small size. Intriguingly, of those ORFs, only approximately 60% (~950 ORFs) were assigned functions. In addition, a considerable number of ORFs were assigned putative functions (Davidsen et al., 2010). Therefore, it is of paramount importance to understand the biological function of these uncharacterized proteins to obtain a more detailed understanding of the pathogenic mechanisms of *H. pylori*.

Earlier studies on pathogenic bacteria showed that expression and function of chaperones are correlated with exposure to various types of stress, including antibiotics, pH fluctuation and oxidative stress (Wolska et al., 2000; Modrzewska et al., 2002; Yamada et al., 2010; Tran et al., 2011). Here, we studied the *H. pylori* ORF named *HP0795* annotated with a hypothetical chaperone function. Despite the availability of its sequence information, little research has been conducted to identify and characterize the HP0795 protein. The aim of this study was to understand the biological function of HP0795. Using genetic and molecular approaches, we demonstrated that HP0795 (a homolog of *E. coli* TF) plays a role as a molecular chaperone, possessing PPIase activity.

2. Methods and materials

2.1. Bacterial strains and growth conditions

H. pylori 26695 was cultivated on Campylobacter agar (CM0935, OXOID) plates supplemented with 5% sheep blood, cefsulodin, vancomycin, trimethoprim and amphotericin B (SR0147E, OXOID) under microaerophilic environment (5% O_2, 10% CO_2 and 85% N_2) at 37 °C.

For *H. pylori* acid-exposure experiments, *H. pylori* 26695 harvested in Campylobacter agar plate for 2 days according to culture condition mentioned above. Afterwards *H. pylori* were resuspended in brucella broth (BD) containing 10% fetal bovine serum (Gibco-BRL) supplemented with by addition of 200 mM HCl until a pH of 3.0 and 5.0, shaking in 200 rpm for 2 h. Acid-exposure and non-exposure *H. pylori* were immediately collected by centrifuge in 4000 rpm, frozen in liquid nitrogen and were subsequently used for isolation of *H. pylori* RNA.

Wild-type *E. coli* BW25113 and *E. coli* mutants were cultured either at 37 °C or 30 °C, with shaking at 220 rpm in Luria-Bertani (LB) medium supplemented with the appropriate antibiotics.

2.2. Bioinformatic analysis

The ClusTALO program in Uniprot website (www.uniprot.org/align) was used to perform comparisons of identity and similar positions between homology sequences of proteins. The secondary structural prediction and formatted multiple sequence alignment were performed using the PSIPRED (bioinf.cs.ucl.ac.uk/pripred) and BoxSHade Server 3.2 (http://www.ch.embnet.org/software/BOX_form.html). The protein subcelluar location was predicted in TargetP 1.1 server (Emanuelsson et al., 2007). The 3-D structure model of HP0795 as a putative protein in *H. pylori* was performed in SWISS-MODEL Repository (Kiefer et al., 2009) and drawn using PyMOL software (www.pymol.org).

Table 1
Primers used in this study.

Primers for HP0795 expression	
HP0795exF:	5′-catatgcatgaatcttgaagtgaaaaagattga-3′
HP0795exR:	5′-ggatccttaacccgcttgaatttttgagc-3′
Primers for RT-PCR	
Hp16sRNAF:	5′-gctctttacgcccagtgattc-3′
Hp16sRNAR:	5′-gcgtggaggatgaaggtttt-3′
HP0795F:	5′-aagggttgaggggcagttgttt-3′
HP0795R:	5′-gcttttctttggctttttcttgac-3′
Primers used to construct strains	
T0795FOR	5′-taagagttgaccgagcactgtgatttttttgaggtaacaagatgaatcttgaagtgaaaaagattga-3′
T0795REV	5′-ggcctttgtgcgaatttagcgcgttatgctgcgtaaattaacccgcttgaatttttgagc-3′
Gtig1	5′-acaccgtctttgcctctcct-3′
Gtig2	5′-atctcgttcgccgctgtat-3′
K-KJA	5′-cagactcacaaccacatgatgaccgaatatatagtggagacgtttagtgtaggctggagctgcttcg-3′
K-KJB	5′-caccctatttttacccaggcctgcccacgggcaggcttttggggaggcatatgaatatcctccttag-3′
GKJ1	5′-agtcaaccgcagtgagtga-3′
GKJ4	5′-cactttacaggtgctcgcat-3′
Primers used to yeast two-hybrid assays	
HP0795BKF	5′-catatgatgaatcttgaagtgaaaaagattga-3′
HP0795BKR	5′-ggatccttaacccgcttgaatttttgagc-3′
HP0921ADF	5′-catatgatgaaaatttttatcaatggatttggccg-3′
HP0921ADR	5′-gaattcttaataatgatacataaactgcgccatatcca-3′

F: forward primer; R: reversed primer. Underlined letters indicate nucleotides added at the 5′end to create a restriction site. AD and BK indicate cloning gene into pGAD or pGBKvector.

2.3. Gene cloning, expression and purification

The *HP0795* fragment (Gene ID: 899053) in *H. pylori* 26695 was amplified by PCR using gene-specific primers pair HP0795exF and HP0795exR (Table 1) containing *Nde* I and *Bam*H I sites of pET28b vector (Novagen) to generate an N-terminal His tag fusion protein. The *E. coli* BL21 cells carrying the resulting plasmids were grown in LB media containing kanamycin at 37 °C until an OD_{600} of 0.7. Expression of HP0795 was induced with 1 mM isopropyl-ß-D-thiogalactopyranoside (IPTG) followed by culturing for 12 h at 18 °C. Cells were centrifuged (4000*g*, 4 °C, 15 min), resuspended in buffer A (20 mM sodium phosphate, 50 mM NaCl, pH 7.8) and passed through a French press. Brocken cells were centrifuged at 15,000*g* (4 °C, 20 min). The HP0795 proteins were collected at buffer B (20 mM sodium phosphate, 50 mM NaCl, 250 mM imidazole, pH 7.8) using immobilized metal affinity chromatography. HP0795 proteins were further dialyzed against phosphate buffer (20 mM sodium phosphate, 50 mM NaCl, pH 7.8) to remove excess imidazole. Purified HP0795 proteins were analyzed using SDS-PAGE on a 10% polyacrylamide gel and visualized after Coomassie blue staining. Concentration of HP0795 was determined using a bicinchoninic acid (BCA) protein assay.

2.4. Crosslinking and electrophoresis

HP0795 at different concentrations was crosslinked using 6 mM disuccinimidylsuberate (DSS, Pierce) dissolved in DMSO at 25 °C in 20 mM sodium phosphate buffer, pH 7.8. Following incubation for 2 h, the crosslinking reaction was stopped by adding 150 mM Tris–HCl, pH 7.0.

The crosslinked products were analyzed by 10% SDS-PAGE and visualized by Coomassie blue staining. The crosslinked and uncrosslinked HP0795 was quantified using ImageJ software