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Characterization of the sensor domain of QseE histidine kinase from *Escherichia coli*

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ABSTRACT

In enterohemorrhagic *Escherichia coli* (EHEC), the QseEF two-component system causes attaching and effacing (AE) lesion on epithelial cells. QseE histidine kinase senses the host hormone epinephrine, sulfate, and phosphate; it also regulates QseF response regulator, which activates LEE gene that encodes AE lesion. In order to understand the recognition of ligand molecules and signal transfer mechanism in pathogenic bacteria, structural studies of the sensor domain of QseE of *Escherichia coli* should be conducted. In this study, we describe the overexpression, purification, and structural and biophysical properties of the sensor domain of QseE. The fusion protein had a 6×His tag at its N-terminus; this protein was overexpressed as inclusion bodies in *E. coli* BL21 (DE3). The protein was denatured in 7M guanidine hydrochloride and refolded by dialysis. The purification of the refolded protein was carried out using Ni-NTA affinity column and size-exclusion chromatography. Thereafter, the characteristics of the refolded protein were determined from NMR, CD, and MALS spectroscopies. In a pH range of 7.4–5.0, the folded protein existed in a monomeric form with a predominantly helical structure. ^{1}H-^{15}N HSQC NMR spectra shows that approximately 93% backbone amide peaks are detected at pH 5.0, suggesting that the number of backbone signals is sufficient for NMR studies. These data might provide an opportunity for structural and functional studies of the sensor domain of QseE.

1. Introduction

Quorum sensing is also cell-to-cell communication in bacteria. It is a critical process for cell survival as it protects the cells from external environment through biofilm formation, virulence, and antibiotic resistance [1–3]. In this process, there is sensing of small molecules, such as oligopeptides in Gram-positive bacteria; AI-1 (autoinduer-1) and AI-2 (autoinducer-2), which are N-Acyl Homoserine Lactones (AHL) found in both Gram-negative and Gram-positive bacteria; and AI-3 (autoinducer-3) in Gram-negative bacteria [1]. AI-1 is synthesized by LuxI and released into the surrounding environment. When the population density of bacteria is high, the local concentration of AI-1 increases in the environment. Consequently, AI-1 diffuses into the cell. Then, LuxR easily senses AI-1 in the cell and activates the luciferase operon [4–6]. In *Escherichia coli* and *Salmonella typhimurium*, AI-2 is created from LuxS-derived 4,5-dihydroxy-2,3-pentanedione (DPD). Then, it is imported into the cell by LsrABC transporters. Finally, it is phosphorylated by LsrK. The phosphorylated AI-2 interacts with the transcriptional repressor, LsrR, which activates *lsr* operon [7]. In *Vibrio* spp, AI-2 (a furanosyl borate diester) binds with LuxP in the periplasm: the resultant complex interacts with the histidine kinase LuxQ. Consequently, there is phosphorylation of conserved histidine of LuxQ. The phosphate group is transferred to the response regulator LuxO *via* the LuxQ/LuxO phoshporyl cascade [1]. Recently, it has been proved that QseBC and QseFE are the two component systems that are involved in bacterial quorum sensing and regulation of virulence factors in pathogenic bacteria, such as enterohemorrhagic *E. coli* (EHEC) O157:H7 and *salmonella enterica* serovar Typhimurium [8–12]. QseC HK senses the auto inducer 3 (AI-3), epinephrine, and norepinephrine of host hormones. Its cognate response regulator QseB regulates virulence factors, including the locus of enterocyte effacement (LEE) genes; these

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genes cause intestinal colonization by stimulating the formation of 'attaching and effacing' (AE) lesions, flagellar, and motility genes [13]. The sensor domain of QseE not only senses epinephrine hormone but also phosphate and sulfate ions. Thereafter, it activates its response regulator QseF [9,10,12], which subsequently regulates the transcription of virulence factor genes, such as *sipA* and *sopB* genes; these genes are involved in the invasion of epithelial cells and *espFu* gene, driving the polymerization of actin during AE lesion formation [11,12]. This indicates that the sensor domains of QseC and QseE may be antimicrobial drug targets that block bacterial quorum sensing and virulence factors [14]. Recently, we have successfully elucidated the structural characteristics of the sensor domain of QseC in solution state [15]. In this study, we produced and characterized the sensor domain of QseE of *E. coli* K12 strain, which has 99% sequence identity from that of EHEC O157:H7 strain. Thus, this study provides a foundation for structural and functional studies that focus on the sensor domains of QseE histidine kinases.

2. Materials and methods

2.1. Cloning and expression of the sensor domain of QseE

The DNA sequence of the sensor domain of QseE (resiudes Q35-Q173) was amplified from genomic DNA of *E. coli* K12 using the polymerase chain reaction (PCR): 5′-CGCCATATGCAAAGCCTGAATGCGCTTAGCG-3′ was used as the forward primer, while 5′-CGCCTCGAGTCATTGCCCACGTTCGGCGATTTCA-3′ was used as the reverse primer. The PCR fragment was cloned into pET28a (+) vector using T4 ligase and the restriction enzymes: *Nde* I and *Xho* I. The plasmid encoded the QseE sensor domain, including 6×His-tag at its N-terminus; this plasmid was transformed into *E. coli* DH 10B competent cells and sequenced. The correct plasmids were transformed into *E. coli* BL21 (DE3) to bring about the expression of the sensor domain of QseE. The cells were grown overnight in 50 mL of Luria-Bertani (LB) broth at 37 °C. Thereafter, 10 mL of a preculture was inoculated into 1 L of LB broth for detecting unlabeled protein or 1 L of minimal media supplemented with $^{15}NH_4Cl$ for detecting ^{15}N labeled protein in a 2.5 L baffled flask. In this flask, the cells were grown at 37 °C until an OD_{600} of 0.6–0.8 was achieved. The proteins were induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG), and the cells were harvested by centrifugation at 3000*g* for 30 min at 4 °C after allowing their growth for 4 h. All the growth media were supplemented with 50 mg/mL kanamycin.

2.2. Refolding and purification of the sensor domain of QseE

The cells harvested from the 1-L culture were resuspended in 20 mL of lysis buffer (20 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), and 2 mM phenylmethylsulfonyl fluoride (PMSF). Then, these cells were lysed in a sonicator. After centrifugating the suspension at 10,000 g for 30 min, the insoluble inclusion bodies were washed thrice with the lysis buffer in a homogenizer. The inclusion bodies from 1-L culture were solubilized in 20 mL of phosphate-buffered saline (PBS) buffer (pH = 7.4), containing 7 M guanidine hydrochloride and 10 mM dithiothreitol (DTT). Following incubation for two hours at room temperature, insoluble cell debris and the lipid membranes were removed by performing high speed centrifugation at 200,000 g for one hour. The solubilized proteins (5 mg/mL) were maintained at a uniform temperature of −80 °C. For refolding the denatured sensor domain of QseE, 10 mg of denatured proteins was diluted in 100 mL of a solution containing the following reagents: 6 M guanidine hydrochloride, PBS buffer (pH = 7.4–7.6), and 5 mM DTT. Then, it was dialyzed four times in 2 L of PBS buffer (pH = 7.4) and 5 mM b-mercaptoethanol at 4 °C using a membrane with 6–8 kDa cut-off. After the dialysis, we removed the precipitants by centrifugation. To purify the refolded sensor domain of QseE, 5 mL of Ni-NTA resin was added into the supernatant and incubated for one hour at 4 °C; the medium was slowly stirred during this period. After packing the resin in a gravity column, the resin was washed with 50 mL of PBS buffer (pH = 7.4); the buffer solution also contained 20 mM imidazole and 5 mM β-mercaptoethanol. The protein was eluted with PBS buffer (pH = 7.4), containing 500 mM imidazole and 5 mM β-mercaptoethanol. While performing overnight dialysis at 4 °C for 20 h in 50 volumes of PBS buffer containing 5 mM β-mercaptoethanol using a dialysis membrane with a molecular weight cutoff of 6–8 kDa (Spectra/Por, Spectrum Labs), the N-terminal 6×His-tag was cleaved by thrombin digestion. Finally, the sensor domain of QseE was obtained by size-exclusion chromatography using a prep-grade HiLoad 16/60 Superdex 75 column (GE Healthcare). Pure protein was stored at a constant temperature of −80 °C for further use.

2.3. Circular dichroism (CD) spectroscopy

CD spectra were acquired in a cylindrical quartz cell (path length = 1 mm) using a J-710 spectropolarimeter (JASCO). Data were acquired at a scan rate of 0.2 nm/s; the average of the data acquired from five scans was determined. The sensor domain of QseE was used at a concentration of 30 μM.

2.4. NMR spectroscopy

NMR measurements were performed with Avance II 800 MHz (Bruker): the instrument was equipped with a cryogenic triple resonance probe. The 2D ^{1}H-^{15}N HSQC spectra of 0.1 mM ^{15}N-labeled protein were obtained in various buffer conditions. All the NMR spectra were processed with Bruker Topspin 3.0.

2.5. Multi-angle light scattering (MALS)

The molecular weights of the sensor domain of QseE were determined by performing high-performance liquid chromatography (HPLC, Shimadzu) and multi angle light scattering (MALS) (Wyatt Technology Corporation) spectrometer. The HPLC instrument was coupled with MALS spectrometer and an integrated technique of analysis was conducted. HPLC was performed with 120 μl of 1 mg/mL of protein: a flow rate 0.5 mL/min was maintained in a WTC-0305S column (Wyatt Technology Corporation). The differential refractive index spectra were obtained using Optilab T-rEX (Wyatt Technology Corporation); this instrument was combined with HPLC and MALS for the purpose of analysis. The molecular weight of protein was calculated using Astra 6.0 software (Wyatt Technology Corporation); a differential refractive index increment (dn/dc) value of 0.185 was used.

3. Results

3.1. Domain organization of QseE and secondary structure prediction of its sensor domain

Domain analysis was conducted using SMART (Simple Modular Architecture Research Tool) service. The results of domain analysis indicated that full length QseE consists of a periplasmic sensor domain (residues 35–174) and a kinase domain. The periplasmic sensor domain is located between two transmembrane helices (residues 15–34 and 175–197), while the kinase domain is composed of two subdomains (His Kinase A (phosphoacceptor) domain and ATP binding CA domain) (Fig. 1A). The secondary structure prediction was based on the composition of amino acid