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Molecular cloning, expression and functional characterization of the 40-kDa heat shock protein, DnaJ, from *Bacillus halodurans*

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ABSTRACT

In the present study, we identified, cloned and expressed a 40-kDa heat shock protein, DnaJ, from *Bacillus halodurans*. The open reading frame of the cloned gene contained 1116 bp and encoded 371 amino acid residues. The purified recombinant DnaJ contained a His-tag at the C-terminus and showed a single band at approximately 41-kDa on SDS-PAGE gel. The 3D structures of DnaJ obtained by I-TASSER showed that the overall structures of DnaJ from *B. halodurans* Guj1 and *E. coli* are very similar, with 45% sequence similarity. The present study revealed that the DnaJ protein from *B. halodurans* inhibits the heat-induced aggregation of insulin in a concentration-dependent manner as aggregation of the insulin B-chain was reduced by approximately 50% at 40 °C in the presence of 0.1 mg/ml of purified recombinant DnaJ. The overexpression of DnaJ improved thermotolerance properties in *E. coli* transformed with pET-28a + DnaJ. Salt resistance experiments indicated that the survival of *E. coli* transformed with DnaJ was enhanced 1.85-fold compared to that of the control cells in the presence of 0.5 M NaCl for 72 h. According to the results obtained, DnaJ from *B. halodurans* can potentially be used for improving the functional properties of enzymes and proteins in various applications.

1. Introduction

Under stress conditions many cellular proteins lose their native three-dimensional structure and function. Many chaperones, called heat shock proteins (HSPs), are proteins that assist nascent protein folding and function in a broad range of structural functional organization of substrate proteins and are upregulated under stress conditions such as elevated temperature, salt and oxidative stress [1–3]. HSPs protect proteins against aggregation; solubilize primary proteins, and help in the folding of nascent proteins, the refolding and degradation of damaged or non-native protein, the assembling of multi-subunit proteins, polypeptide transport across biological membranes, and proteolysis [4–8]. According to their nature of functions and molecular mass, HSPs are classified broadly into six major families: high-molecular-mass HSPs (≥100 kDa), HSP90 (81–99 kDa), HSP70 (65–80 kDa), HSP60 (55–64 kDa), HSP40 (35–54 kDa), and small HSPs (≤34 kDa) [9]. DnaJ, also known as the J-protein and is the homologue of the eukaryotic HSP40, is a cofactor of DnaK. This protein was first identified in *E. coli* with a mass of approximately 44-kDa [10–12] as a co-chaperone protein that interacts with DnaK HSP (HSP70 in eukaryotes) and up-regulates its ATPase activity, thus regulating the binding capacity of DnaK to substrate proteins [13]. DnaJ contains four modules including a highly conserved J-domain of approximately 70 amino acids at the N-terminal, and a glycine/phenylalanine –rich region (G/F domain) of approximately 30 residues. This region is essential for DnaJ interactions with DnaK. Following this is a zinc finger like domain containing four CXXCXGXG motifs for interaction with others proteins and a C-terminal region to bind substrate proteins [14–17]. A conserved motif with histidine- proline-aspartic acid (HPD) in the J-domain is required for accelerating the ATPase activity of HSP70 [18]. Although DnaK has a high affinity for misfolded proteins, it does not bind to polypeptides directly [19]. DnaJ is known to recognize and bind to the hydrophobic segments of the unfolded polypeptide and then transfer it to DnaK, thus maintaining their solubility and preventing aggregation [20,21]. DnaJ is a major component of the DnaK chaperone system that protects bacteria in harsh environments [18]. Disassociation of DnaK and DnaJ from the polypeptide is facilitated by GrpE, which catalyzes the exchange of guanine nucleotide [19]. DnaJ was originally shown to be involved in phage replication and DNA transcription initiation. Previous studies have recognized that J-domain mutations, lead to functional defects in DNA and RNA synthesis, protein degradation and cell division. In general, the DnaK-DnaJ molecular chaperone complex

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Table 1
Primers sequence used in this study.

Primer	Sequence(5′→ 3′)
DnaJ-F	GGTTGTGGATCCATGAGTAAACGAGACT
DnaJ-R	TTGTTACTCGAGTTCCCCTTTGAAAGC

Restriction sites (*BamHI* and *XhoI*) are underlined in the sequences.

plays a critical role in the structural-functional organization of cellular proteins. Identification, purification and characterization of novel chaperones from new extremophile sources are very important for biotechnological and industrial applications. This study describes the identification, molecular cloning, expression, purification and characterization of a novel DnaJ from *Bacillus halodurans* Guj1. In addition, to examine the chaperone-like activity of purified DnaJ, bovine pancreatic insulin was used as a target protein.

2. Materials and methods

2.1. Strains, plasmids and culture conditions

B. halodurans Guj1 was obtained from the Iranian Biological Resource Center and was used for genomic DNA extraction. For the expression of the DnaJ gene, two expression systems were used. *E. coli* DH5α with plasmid pTZ57R/T (Thermo Scientific, Waltham, MA, USA) and *E. coli* BL21 with plasmids pET28a (Novagen, USA). Luria-Bertani (LB) broth (1.0% tryptone, 0.5% yeast extract and 0.5% NaCl) and LB agar (1.0% tryptone, 0.5% yeast extract, 0.5% NaCl and 1.5% agar) were used as media, and X-gal (40 mg/ml), isopropyl β-D-1-thiogalactopyranoside (IPTG) (100 mg/ml), ampicillin (100 mg/ml) and kanamycin (50 mg/ml) were used for the preparation of growth media.

2.2. Cloning of the DnaJ gene from B. halodurans

The genomic DNA of *B. halodurans* was extracted using QIAamp DNA mini kit (QIAGEN, Germany). Primers were designed against DnaJ of from *Bacillus cereus* FORC-005 and *Bacillus thuringiensis* BGSC 4AA1 (GenBank accession number CP009686.1 and CP010577.1 respectively) and used to amplify the DnaJ gene from *B. halodurans* (Table 1). PCR was performed for 35 cycles of denaturation (40 s at 94 °C), annealing (30 s at 54 °C), and extension (1 min at 72 °C) with 5 min final extension at 72 °C. PCR products were resolved in 1.5% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. DNA bands in the expected size were excised from the gel and purified using the QIAquick Gel Extraction Kit (GIAGEN, Germany). The extracted fragment was cloned into pTZ57R/T vector and transformed into chemically competent *E. coli* DH5α. *E. coli* cells were plated onto LB agar plates containing ampicillin, and X-gal and incubated at 37 °C for 15–18 h for confirmation of transformation. After confirmation, one clone was selected and sub-cultured in LB broth at 37 °C overnight. The sequence of the cloned DNA fragment was confirmed by sequence analysis (Macrogen, South Korea). The gene sequences were identified by a BLAST search of databases at National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). Amino acid sequences of DnaJ from a group of *Bacillus* spp and *E. coli* were compared using the ClustalW2.0.12 Program in the EMBL-EBI database (http://www.ebi.ac.uk). The deduced protein sequence of DnaJ was analyzed for functionally important regions using Conserved Domain Search program (CDD v2.15, NCBI), and protein sequence was analyzed using Compute pI/Mw tool to calculate the theoretical isoelectric point (pI) and protein molecular weight (Mw) (http://www.expasy.org/tools/).

2.3. Plasmid construction

BamHI and *XhoI* restriction sites were introduced into the forward primer (DnaJ-F2) and reverse primer (Danj-R2), respectively (Table 1). PCR was performed using the following amplification program: 4 min at 95 °C, 40 s at 95 °C, 30 s at 58 °C, 1 min at 72 °C (30 cycles), and finally 5 min at 72 °C. The pET-28a vector and PCR products were digested with *BamHI* and *XhoI*. After ligation, the generated pET-28a + DnaJ, which encodes DnaJ protein with a C-terminal His-tag, was transformed into competent *E. coli* BL-21 cells.

2.4. Expression and purification of the His tagged DnaJ protein

BL21 cells harboring the DnaJ expression plasmid were cultured overnight in LB medium supplemented with 50 μg/ml kanamycin at 37 °C and 120 rpm. DnaJ expression was achieved by inducing of the cultures with 1 mM IPTG, and the cells were incubated at different times ranging from 2 to 10 h at 37 °C. Following cell lysis by sonication (4 pulses of 30 s, interspersed with cooling on ice), cell debris was pelleted by centrifugation at 3000 rpm for 20 min, and clear supernatants were obtained. The supernatant was loaded onto columns to purify the protein by Ni-NTA agarose (QIAGEN, Germany). The unbound and weakly bound proteins were washed off using a wash buffer (50 mM imidazole, 5 mM Tris, 300 mM NaCl, pH 7.5), and the His-tagged DnaJ was eluted using an elution buffer (300 mM imidazole, 5 mM Tris, 300 mM NaCl, pH 7.5). Protein homogeneity was confirmed by 12.5% (w/v) SDS-PAGE.

2.5. Thermotolerance experiments

The thermotolerance properties of *E. coli* cells transformed with DnaJ were determined as described before [22]. For the thermotolerance assays, 1 mM of IPTG was added to mid-log phase cultures (OD_{600} = 0.6), and the transformed *E. coli* cells (pET-28a + DnaJ) were incubated at 37 °C for 3 h; Subsequently, the cells were subjected to thermal stress at 45 °C, 48 °C, and 51 °C for various periods of time (0, 15, 30, 60 and 120 min). These heat-treated cells were spotted on LB plates containing 50 μg/ml of kanamycin and were incubated overnight at 37 °C. For measuring the survival rate, the percentage of surviving *E. coli* colonies (pET-28a + DnaJ) were calculated and compared with control *E. coli* cells (pET-28a without DnaJ) cultured under same conditions.

2.6. NaCl-stress treatments

To determine whether DnaJ could enhance the salt tolerance of *E. coli*, the effect of NaCl-stress on the growth of *E. coli* cells transformed with DnaJ was examined. For the salt tolerance assays, the transformed *E. coli* cells (pET-28a + DnaJ) were cultured at 37 °C for 3 h, and 1 mM of IPTG was added to mid-log phase cultures. Subsequently, 4.0×10^8 cells/ml from each sample were plated on LB agar containing 0.5 and 1 M NaCl and 50 μg/ml of kanamycin. The plates were then incubated at 37 °C for various periods of time (48, 60, 72 and 84 h). Cell viability was determined by counting the number of colony-forming units. Survival rates of the two samples (*E. coli* with and without pET-28a + DnaJ) were compared. Cell viability was calculated according to the following formula:

$$\%\text{Survival rate} = \text{Colony numbers on stressed plate} / \text{Colony numbers on control plate} \times 100$$