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ORIGINAL ARTICLE

Optimization of expression and purification of HSPA6 protein from *Camelus dromedarius* in *E. coli*

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Hsp70;
Molecular chaperone

Abstract The HSPA6, one of the members of large family of HSP70, is significantly up-regulated and has been targeted as a biomarker of cellular stress in several studies. Herein, conditions were optimized to increase the yield of recombinant camel HSPA6 protein in its native state, primarily focusing on the optimization of upstream processing parameters that lead to an increase in the specific as well as volumetric yield of the protein. The results showed that the production of cHSPA6 was increased proportionally with increased incubation temperature up to 37 °C. Induction with 10 μM IPTG was sufficient to induce the expression of cHSPA6 which was 100 times less than normally used IPTG concentration. Furthermore, the results indicate that induction during early to late exponential phase produced relatively high levels of cHSPA6 in soluble form. In

Abbreviations: amp, ampicillin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid chromatography; IPTG, isopropyl β-D-1-thiogalactopyranoside; LB, Luria–Bertani; 2× LB, double strength Luria–Bertani; NB, nutrient broth; Ni–NTA, nickel–nitrilotriacetic acid; OD_{600}, optical density at 600 nm; PMSF, phenylmethylsulfonyl fluoride; rpm, rotations per minute; TB, terrific broth

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addition, 5 h of post-induction incubation was found to be optimal to produce folded cHSPA6 with higher specific and volumetric yield. Subsequently, highly pure and homogenous cHSPA6 preparation was obtained using metal affinity and size exclusion chromatography. Taken together, the results showed successful production of electrophoretically pure recombinant HSPA6 protein from *Camelus dromedarius* in *Escherichia coli* in milligram quantities from shake flask liquid culture.

1. Introduction

Heat shock proteins (molecular chaperones) belong to a very essential group of proteins involved in the stability and control of structure–function of proteins under unfavorable conditions. Heat shock activity was first discovered in Drosophila in response to high temperature (Ritossa, 1962). Under thermal stress, the protein activity dramatically increased at transcription level (Wu, 1995). It has previously been observed that the open reading frames and regulatory sequences of heat shock genes are highly conserved (Lindquist, 1986). Research on different heat shock protein classes in various organisms indicated that heat shock genes are induced by many environmental factors such as high temperature, osmotic stress, oxidative stress, heavy metals, alcohol, damaged/aggregated proteins, exposure to toxins, different deficiencies and diseases (Jakob et al., 1999; Spiess et al., 1999; Santoro, 2000; Foit et al., 2009; Quan et al., 2011; Quan and Bardwell, 2012). Arabian camel (*Camelus dromedarius*) successfully survives under extremely stressful environment of heat and osmotic stresses. The cellular factor that helps in attaining a three-dimensional structure of the proteins and protects them from inactivation and misfolding during extremely hot and dry summer days in camel is largely unknown.

HSPA6 also known as HSP70B' (70 kDa), has been involved in maintaining cellular proteostasis (Heldens et al., 2010). The mRNA of HSPA6 was found to be significantly increased at transcription level under different stress conditions and could be used as a useful biomarker (Leung et al., 1990; Xu et al., 2000; Wada et al., 2005; Wang et al., 2005; Noonan et al., 2007). Both, HSPA6 and Human HSP72 are evolutionarily related but unlike HSP72, HSPA6 is stringently inducible, resulting in no significantly detectable level in most cells (Leung et al., 1990; Parsian et al., 2000).

The camel's HSPA6 nucleotide and amino acid sequences have shown a high degree of similarity with other mammalian species. Moreover, the predicted three dimensional structure of camel HSPA6 showed 87% and 82% similarity with human and mouse HSPs (Elrobh et al., 2011). However, detailed biophysical, structural and functional studies using pure camel heat shock proteins are lacking. To perform such studies, highly pure homogenous preparations of a protein in milligram quantity are needed. Keeping this in mind, this study was aimed at expressing, optimizing and producing a large quantity of pure recombinant cHSPA6 in *Escherichia coli*. We have selected *E. coli* to express the recombinant cHSPA6 because it has been proven to be a very good host for the heterologous expression of recombinant proteins, and for many purposes *E. coli* is the best host (Baneyx, 1999; Pines and Inouye, 1999; Lebendiker and Danieli, 2014; Rosano and Ceccarelli, 2014). The recent advances in physiology and genetics of the *E. coli* at molecular level offer a great opportunity for the rapid and economical production of recombinant proteins (Malik et al., 2006). It has previously been shown that genetically fused purification tag enhances the level of purity and protein recovery (Malik et al., 2006, 2007). Here in this study, we have optimized various growth parameters (cultivation temperature, inducer concentration, culture media, pre- and post-induction incubation), we were successful in obtaining highly pure cHSPA6 in milligram quantities from shake flask experiments.

2. Materials and methods

2.1. Chemicals and instruments

The ORF of cHSPA6 cloned on pET15 vector and expression host *E. coli* BL21 (DE3) pLysS were kindly provided by Elrobh et al. (2011). IPTG and ampicillin were obtained from Biobasic. Benzonase was purchased from Sigma, Chicken egg lysozyme from USB Corporation. Superdex 75, Ni–NTA resin, low molecular weight markers and prepacked columns were from Amersham Biosciences. All other chemicals used in this study were of reagent grade. Ultrospec 2100 pro Spectrophotometer, AKTA purification system, SDS–PAGE assembly were from Amersham Biosciences. Thermomixer, electroporator and benchtop cooling centrifuge were from Eppendorf. Lamp sterilizer from Cole-Parmer, shaking incubator from Jeio Tech, South Korea, gel scanner from Epson and pH meter was from Sentron.

2.2. Expression of cHSPA6 in E. coli

In this study, *E. coli* BL21 (DE3) pLysS was used for expression of cHSPA6. 1 µl of pET15-cHSPA6 plasmid was transformed into high efficiency chemical competent cell of *E. coli* BL21 (DE3) pLysS (Swords, 2003; Sambrook and Russell, 2006). We have used 200 µg/ml ampicillin in the solid and liquid medium to maintain plasmid throughout this study. Glycerol stocks of the transformed cells were made by mixing 1:1 ratio of the freshly grown overnight culture with 30% (w/v) sterilized glycerol solutions and stored at −80 °C. Single colony of *E. coli* BL21 (DE3) pLysS strain harboring pET15-cHSPA6 plasmid was inoculated into 20 ml LB_{amp} grown overnight in shaking incubator at 37 °C. Pre-inoculum (0.2 ml) was inoculated into 20 ml LB_{amp} and culture was grown at 37 °C until OD_{600} was reached 0.65. The expression of cHSPA6 was induced with isopropyl β-D-thiogalactopyranoside (IPTG) as described (Studier et al., 1990). In the preliminary expression experiment, culture at the mid exponential phase was induced with 1 mM IPTG. The culture was grown for 3 h post induction at 37 °C, 200 rpm. Before harvesting culture, final growth of the culture was calculated by measuring OD at 600 nm. To harvest, 1 ml