A 70-kDa molecular chaperone, DnaK, from the industrial bacterium Bacillus licheniformis: gene cloning, purification and molecular characterization of the recombinant protein

Wan-Chi Liang · Xuan-Hui Wang · Min-Guan Lin · Long-Liu Lin

Received: 2 October 2008 / Accepted: 27 November 2008

Abstract The heat shock protein 70 (Hsp70/DnaK) gene of Bacillus licheniformis is 1,839 bp in length encoding a polypeptide of 612 amino acid residues. The deduced amino acid sequence of the gene shares high sequence identity with other Hsp70/DnaK proteins. The characteristic domains typical for Hsps/DnaKs are also well conserved in B. licheniformis DnaK (BlDnaK). BlDnaK was overexpressed in Escherichia coli using pQE expression system and the recombinant protein was purified to homogeneity by nickel-chelate chromatography. The optimal temperature for ATPase activity of the purified BlDnaK was 40°C in the presence of 100 mM KCl. The purified BlDnaK had a Vmax of 32.5 nmol Pi/min and a KM of 439 μM. In vivo, the dnaK gene allowed an E. coli dnaK756-ts mutant to grow at 44°C, suggesting that BlDnaK should be functional for survival of host cells under environmental changes especially higher temperature. We also described the use of circular dichroism to characterize the conformation change induced by ATP binding. Binding of ATP was not accompanied by a net change in secondary structure, but ATP together with Mg2+ and K+ ions had a greater enhancement in the stability of BlDnaK at stress temperatures. Simultaneous addition of DnaJ, GrpE, and NR-peptide (NRLLLTG) synergistically stimulates the ATPase activity of BlDnaK by 11.7-fold.

Keywords Bacillus licheniformis · DnaK · ATPase activity · Escherichia coli · Circular dichroism

Introduction

A sudden increase in temperature and other types of environmental stresses induce the synthesis of a specific set of proteins called heat shock proteins (Hsps). The first description of a subset of cellular proteins in Drosophila induced by heat shock [1] triggered extensive studies on their function in stress tolerance in a variety of organisms. Hsps are classified into five major families on the basis of their molecular size in kilodaltons (kDa): Hsp110, Hsp90, Hsp70, Hsp60 and the low-molecular weight Hsp family. The Hsp70/DnaK family occurs in diverse organisms and members of this family play an essential role in protein metabolism in both stressed and unstressed organisms. Hsp70/DnaK proteins are involved in de novo protein folding, membrane translocation, formation and disassembly of protein complexes and degradation of misfolded proteins [2–4].

Hsp70s/DnaKs are composed of a highly homologous N-terminal ATPase domain, a substrate-binding domain and a C-terminal variable domain [5]. The molecular chaperone function of Hsp70/DnaK proteins seems to be based on its ATPase activity and this activity is stimulated by Hsp40/DnaJ, GrpE and substrate binding [6]. The chaperone activity of Hsp70/DnaK proteins is dependent on a close interaction between the substrate-binding domain and the ATPase domain. When ATP is bound to the ATPase domain of DnaK, there is poor substrate binding and hence it exhibits a weak chaperone activity. However, there is high affinity for substrate and the increase in the chaperone

W-C Liang1 · X-H Wang1 · M-G Lin2 · L-L Lin1 (✉)
1Department of Applied Chemistry,
2Department of Biochemical Science and Technology, National Chiayi University, 300 University Road, Chiayi - 60083, Taiwan
E-mail: llin@mail.ncku.edu.tw
activity when ADP is bound. The ATPase activity is regulated by the co-chaperone protein DnaJ [7, 8]. The DnaJ proteins stimulate the low basal ATP hydrolysis activity of partner Hsp70s/DnaKs [6,9] and in doing so enhance the Hsp70s/Dnak substrate-binding activity and hence their chaperone activity.

*Bacillus licheniformis* is a gram-positive, spore-forming bacterium widely distributed as a saprophytic organism in the environment. Unlike most other bacilli, which are predominantly aerobic, *B. licheniformis* is a facultative anaerobe, which may allow it to grow in additional ecological niches. There are numerous commercial and agricultural uses for *B. licheniformis* and its extracellular products. The species has been used for decades in the manufacture of industrial enzymes including several proteases, α-amylase, penicillinase, pentosanase, cycloglucosyltransferase, β-mannanase and several pectinolytic enzymes. The proteases from *B. licheniformis* are used in the detergent industry as well as for dehairing and bating of leather [10, 11]. Additionally, amylases from *B. licheniformis* are deployed for commodity scale production of sugars from starches [12]. Specific *B. licheniformis* strains are also used to produce the peptide antibiotic bacitracin as well as a number of specialized chemicals such as γ-polyglutamate [13–16].

Molecular chaperones have a potential role in biotechnology to enhance the recombinant over-expression of a particular protein of interest [17]. To date, the majority of works that have been done on defined DnaK-DnaJ systems at a prokaryotic level has principally involved in the *E. coli* system [18–22]. A number of works have also been performed on other prokaryotic organisms [23–29]. Given that *B. licheniformis* is an industrial organism used for the manufacture of enzymes, antibiotics, and chemicals, the complete nucleotide sequence of the type strain (*ATCC 14580*) has been determined [30]. With the release of the genome sequence, it has become straightforward to isolate the interesting genes. In this study, we demonstrate the successful expression, over-production and purification of the *B. licheniformis* DnaK protein (*Bld*DNAK). Also, the purified protein was characterized in the aspects of optimal temperature and pH, effects of metal ions and the conformational changes induced by nucleotide binding. The *Bld*DNAK protein was shown by in vivo and in vitro means to have the properties of a molecular chaperone.

### Materials and methods

#### Materials

**Restriction endonucleases,** T4 DNA ligase, *Taq* DNA polymerase, and DNA molecular-mass markers were from Promega Life Sciences (Madison, WI, USA). Ni²⁺-nitrilotriacetate (Ni²⁺-NTA) resin, QiAprep spin miniprep kit and QIA-quick gel extraction kit were obtained from Qiagen (Valencia, CA, USA). *Escherichia coli* BB2362 (*dnaK756 recA::Tc² pDM1.1*) was kindly provided by Prof. B. Bukau (University of Heidelberg, Germany). Adenosine 5′-triphosphate (ATP) disodium salt was purchased from Merck Ltd. (Darmstadt, Germany). Reagents for polyacrylamide gel electrophoresis (PAGE) were acquired from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals were commercial products of analytical grade or molecular grade.

#### Bacterial strains, plasmids, and growth conditions

*B. licheniformis* ATCC14580 obtained from the Bioresources Collection and Research Center in Food Industry Research & Development Institute (Hsinchu, Taiwan) was used as a source of chromosomal DNA for the cloning of dnaK, dnaJ, and grpE genes. *E. coli* novablue (Novagen, Maidson, WI, USA) was used for the routine preparation and construction of recombinant plasmids. *E. coli* M15 (pRep4) from Qiagen was employed for T5 RNA polymerase-mediated over-expression of recombinant proteins. Plasmids used were pGEM-T Easy vector (Promega) and pQE-30 (Qiagen).

*B. licheniformis* was cultivated in nutrient broth with rotary shaking (150 rpm) at 37°C for 18 h, whereas *E. coli* strains were grown in LB medium at 20°C during isopropyl-β-D-thiogalactopyranoside (IPTG)-induced gene expression. As required, ampicillin and kanamycin were used at a final concentration of 100 and 25 μg/ml, respectively.

#### General molecular techniques

Chromosomal DNA of *B. licheniformis* was isolated according to the method of Doi et al. [31]. Conventional techniques for DNA manipulations such as restriction enzyme digests and agarose electrophoresis were performed as described by Sambrook and Russel [32]. *E. coli* cells were made competent for transformation by the method of Dagert and Ehrlich [33]. DNA sequencing was performed by Mission Biotechnology (Taipei, Taiwan). Amino acid sequences were analyzed with the programs BLAST-X [34] from the National Center for Biotechnology Information (National Library of Medicine, National Institute of Health, MD) and Alignment from the ExPASy molecular biology server (Swiss Institute of Bioinformatics, Basel, Switzerland).

#### Expression and purification of recombinant *Bld*DNAK, *Bld*DNAJ and *Bld*GrpE

The open reading frames of *B. licheniformis* dnaK (EMBL AAU24248.2), dnaJ (EMBL AAU24247.1), and grpE genes were deposited in the Swiss Institute of Bioinformatics.