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Recombinant expression and in vitro refolding of the yeast small heat shock protein Hsp42☆

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Abstract

Small Hsps represent a variation on the theme of protection of proteins from irreversible aggregation by reversible interaction with chaperone proteins. While different sHsps are highly heterogeneous in sequence and size, the common trait is the presence of a conserved α-crystallin domain. In addition sHsps assemble into large oligomeric complexes where dimers represent the basic building blocks. Hsp42, a member of the sHsp family in the cytosol of *S. cerevisiae*, forms ordered oligomers with a barrel-like structure. Here, we present the recombinant expression and purification of Hsp42. We demonstrate, that Hsp42 is expressed in inclusion bodies and can be resolubilized and folded to correct, active oligomers. This indicates that in contrast to thermal unfolding, the chemical disassembly and unfolding of Hsp42 is fully reversible. In comparison to the purification of mature Hsp42 from yeast, its recombinant expression leads to a substantial increase in the yield of the protein and to a reduction of contamination caused by aggregation prone proteins complexed by Hsp42. In addition, the recombinant Hsp42 is fully active as a chaperone in an energy independent manner.

Keywords: Hsp42; sHsp; Protein folding; Protein purification; Chaperone activity

1. Introduction

sHsps show a characteristic heterogeneity in sequence and size. The common trait is the conserved C-terminal domain, the so-called α-crystallin domain. The term refers to the most prominent family member, the eye-lens protein α-crystallin [1]. However, all sHsps investigated up to now form large oligomeric complexes, mainly of 12–42 subunits [2–6]. Structural analysis of several members of the family revealed hollow particles with openings, where the basic building block of the oligomer is a dimer [7–11]. Several species specific variations of this common scheme have been described recently. For example, wheat Hsp16.9 assembles into a dodecameric double disk, each disk organized as a trimer of dimers [8]. There also seem to be critical variations in the stability of the oligomers. While the archaeal Hsp16.5 has a rigid and well-defined quaternary structure, subunit exchange and high flexibility of the oligomeric assembly were detected for α-crystallin and other sHsps [11–16]. sHsps have been included in the class of molecular chaperones because they bind specifically to unfolded proteins in vitro and prevent their aggregation by forming large, stable sHsp–substrate complexes of globular shape [9,10,17–21].

Although the non-native substrate protein is not released spontaneously, these complexes are not dead-ends. In the presence of ATP, Hsp70 and/or Hsp104 chaperones are able to promote the folding of the bound substrate proteins to the native state [18,19,22–25]. In vivo the emerging picture is that sHsps bind non-native proteins under stress conditions. Binding prevents the formation of aggregates and enables the subsequent refolding by Hsp70, Hsp104 or other ATP-dependent chaperone systems [22–27].

The cytosol of *S. cerevisiae* contains at least two chaperones of the sHsp family with partially overlapping function. Hsp42 acts as a chaperone both at physiological and heat shock

Abbreviations: Rho, Rhodanese; BSA, bovine serum albumin; DTT, dithiothreitol; (s)Hsp, (small) heat shock protein; SEC, size exclusion chromatography; PAGE, polyacrylamide gel electrophoresis; OD, optical density; EDTA, ethylenedinitrilotetraacetic acid

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temperatures [10], while Hsp26 is significantly activated as a chaperone at elevated temperatures [9]. Together they prevent aggregation of cytosolic proteins under physiological and stress conditions.

Like α-crystallin and other sHsps, Hsp42 is a dynamic oligomer and single particle analysis of negative-stain preparations revealed a predominant population of ring-like complexes [10]. The averaged projection image of Hsp42 strongly resembles the structural organization of Hsp16.9 from wheat [8], a double disc of two hexameric rings.

Hsp42 is a promiscuous chaperone. It is able to suppress the aggregation of a broad variety of substrate proteins in vitro and is able to bind at least 30% of the yeast cytosolic proteins [10]. This promiscuousness is one of the major problems in purifying mature Hsp42 from the yeast cytosol. Due to its high substrate binding activity various contaminants are stably bound and hard to separate.

Here we present a strategy to express Hsp42 in *E. coli* and purify it under denaturing conditions. Surprisingly, Hsp42 could be refolded efficiently in vitro, resulting in correctly assembled and functional oligomers. Furthermore, testing the functionality of Hsp42 we were able to show for the first time that Hsp42 does not hydrolyze ATP and, like other sHsps, acts in an energy independent manner.

2. Experimental

2.1. Materials

Rhodanese was obtained from Sigma (St. Louis, USA). The Hsp26 and Hsp104 expression plasmids were a kind gift of Dr. S. Lindquist (Whitehead Institute, Boston, USA). Hsp26 and Hsp104 were expressed and purified according to Haslbeck et al. [9] or Schirmer and Lindquist [28], respectively. For comparison, native Hsp42 was purified from yeast as described elsewhere [10]. For ATPase activity measurements, mature Hsp42 was further purified in analytical scale on a 0.2 ml ATP agarose column, where Hsp42 was found in the flow through, followed by an additional size exclusion chromatography step (buffer, 100 mM Tris/HCl, 150 mM NaCl, 1 mM DTT, pH 8.0; TSK 4000 PW; TosoHaas, Stuttgart, Germany). Densitometric analysis of Coomassie stained SDS-PAGE gels was performed using the ImageMaster 1D software (Amersham Biosciences, Freiburg, Germany).

2.2. Cloning and expression of recombinant Hsp42

HSP42 was amplified from genomic yeast DNA via PCR with the primers GACTGGATCCAGTTTTTATCAACAATCCCTATCTC, CATGAAGCTTTCAATTTTCTACCGTAGGGTTGG and cloned into pQE60 (Qiagen, Hilden, Germany) via the introduced *Bam*HI and *Hin*dIII restriction sites. The protein was expressed in *E. coli* M15 (Qiagen, Hilden, Germany) cultivated at 37 °C. Induction of the expression plasmids was performed at $OD_{600} = 0.5–0.8$ with 2 mM IPTG shifting the cells to 30° subsequently after induction.

2.3. Isolation and solubilization of inclusion bodies

The cell pellet was resolved in 50 mM Tris/HCl, 1 mM EDTA, 5% glycerol, pH 7.4, and incubated with 1.5 mg of lysozyme/g of cells for 30 min at 4 °C. Cells were lysed with a disrupter (Constant Systems). The lysed cells were incubated with 10 μg of DNase I and 3 mM $MgCl_2$ for 30 min at 25 °C to digest the DNA. To isolate the inclusion bodies 0.5 volumes of 60 mM EDTA, 6% Triton X-100, 1.5 M NaCl, pH 7.0 were added and the solution was incubation for 30 min at 4 °C. The inclusion body pellet was centrifuged (40,000 × *g*, 30 min, 4 °C) and washed in 50 mM Tris/HCl, 20 mM EDTA, pH 7.4. The pellet was resolubilized in 50 mM Tris/HCl, 8 M urea, 100 mM DTT, pH 7.4, and incubated for 2 h at 25 °C.

2.4. Chromatography

All chromatography steps were performed on an Äkta FPLC system (Amersham Biosciences, Uppsala, Sweden). Ion exchange chromatography was performed using five column volumes (CV) for equilibration, loading of sample, 5–10 CV for washing and linear gradients of 15–25 CV. Buffer conditions, flow rates and column types are stated in results.

2.5. Electrophoresis

SDS-PAGE was performed in a SE 250 Mighty Small electrophoresis system according to the manufacturers' protocol (Amersham Biosciences, Freiburg, Germany) at a constant current of 30 mA per gel. Coomassie staining was performed as described elsewhere [29].

2.6. Immunoblotting

Proteins were separated by SDS-PAGE, and transferred to PVDF membranes. The immunodetection was performed using affinity-purified polyclonal rabbit antiserum raised against purified Hsp42. For detection, a horse radish peroxidase-linked secondary conjugate (Sigma, St. Louis, USA) was used and reactive bands were visualized by Enhanced Chemiluminescence (ECL) Detection Reagents (Amersham Biosciences, Freiburg, Germany). Alternatively, Cy5 conjugated antibodies against rabbit IgG (Amersham Biosciences, Freiburg, Germany) were used for detection and analysis of Hsp42 quantities [10]. For calculation of protein contents, dilution series of purified Hsp42 were used for calibration.

2.7. Circular dichroism spectroscopy

CD spectra were recorded using a Jasco J-715 spectropolarimeter (Jasco, Groβumstadt, Germany). The experiments were carried out in quartz cuvettes with 0.02 cm path length. Far UV-spectra were recorded from 200 to 250 nm in 50 mM potassium phosphate (pH 7.4) at 20 °C; 16 spectra were accumulated and all spectra were buffer-corrected. Structural elements were calculated using the CDNN software (http://www.bioinformatik.biochemtech.uni-halle.de/cdnn).