doi:10.1016/j.jmb.2006.03.027 *J. Mol. Biol.* (2006) **358**, 1367–1377

JMB

Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

ELSEVIER

Thermal Adaptation of the Yeast Mitochondrial Hsp70 System is Regulated by the Reversible Unfolding of its Nucleotide Exchange Factor

Fernando Moro and Arturo Muga*

Unidad de Biofísica (CSIC/UPV-EHU) y Departamento de Bioquímica y Biología Molecular, Facultad de Ciencia y Tecnología Universidad del País Vasco Apartado 644, 48080 Bilbao Spain

**Corresponding author*

The Hsp70 protein switches during its functional cycle from an ADP-bound state with a high affinity for substrates to a low-affinity, ATP-bound state, with concomitant release of the client protein. The rate of the chaperone cycle is regulated by co-chaperones such as nucleotide exchange factors that significantly accelerate the ADP/ATP exchange. Mge1p, a mitochondrial matrix protein with homology to bacterial GrpE, serves as the nucleotide exchange factor of mitochondrial Hsp70. Here, we analyze the influence of temperature on the structure and functional properties of Mge1p from the yeast *Saccharomyces cerevisiae*. Mge1p is a dimer in solution that undergoes a reversible thermal transition at heat-shock temperatures, i.e. above 37 °C, that involves protein unfolding and dimer dissociation. The thermally denatured protein is unable to interact stably with mitochondrial Hsp70, and therefore is unable to regulate its ATPase and chaperone cycle. Crosslinking of wild-type mitochondria reveals that Mge1p undergoes the same dimer to monomer temperature-dependent shift, and that the nucleotide exchange factor does not associate with its Hsp70 partner at stress temperatures (i.e. ≥45 °C). Once the stress conditions disappear, Mge1p refolds and recovers both structure and functional properties. Therefore, Mge1p can act as a thermosensor for the mitochondrial Hsp70 system, regulating the nucleotide exchange rates under heat shock, as has been described for two bacterial GrpE proteins. The thermosensor activity is conserved in the GrpE-like nucleotide exchange factors although, as discussed here, it is achieved through a different structural mechanism.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: nucleotide exchange factor; Mge1p; GrpE; Hsp70; mitochondria

Introduction

Hsp70 proteins, together with their co-chaperones, participate in a large number of essential cellular processes, such as: (i) refolding of stress-denatured proteins; (ii) folding of newly synthesized proteins; (iii) translocation into and folding inside organelles; and (iv) assembly and disassembly of protein complexes.[1] Their chaperone activity relies on different nucleotide exchange factors that accelerate the conversion rate from the high (ADP-bound) to the low substrate affinity (ATP-bound) conformation. Among the nucleotide exchange factors described so far are: HspBP1, BAG1, and GrpE-like proteins.[2–5] Several structural features within the ATPase domain of Hsp70 proteins control the interaction with exchange factors and the dissociation of nucleotides.[6] The best known nucleotide exchange factor is GrpE from *Escherichia coli*, that acts as an ADP/ATP exchanger for DnaK, the main bacterial Hsp70.[4,7] GrpE has proven to be essential for cell viability at all temperatures.[8] In *Saccharomyces cerevisiae*, a close homolog of GrpE, named Mge1p, was found to be a protein of the mitochondrial matrix essential for cellular life.[9] Deletion of the MGE1 gene (also termed YGE1) leads to accumulation of mitochondrial precursor proteins.[10,11] Mge1p modulates the nucleotide-dependent stability of the mitochondrial import motor formed by mtHsp70 and Tim44,[12,13] and it is required for folding of newly imported precursors.[10] It has been

Abbreviations used: DSC, differential scanning calorimetry; DSS, dissuccinimidyl suberate.

E-mail address of the corresponding author: gbpmuvia@lg.ehu.es

0022-2836/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.

shown that Mge1p acts as a nucleotide exchange factor of mtHsp70, stimulating nucleotide release.[14,15]

Mge1p has approximately 30% sequence identity with GrpE from *E. coli*, the nucleotide exchange factor of bacterial DnaK. The functional identity between both proteins is exemplified by the fact that Mge1p is able to substitute for GrpE in *E. coli*.[16] Moreover, Mge1p can interact with DnaK and assist refolding of denatured firefly luciferase *in vitro*,[17,18] suggesting also a high level of structural similarity. As seen in the crystal structure of GrpE complexed with the DnaK ATPase domain,[19] the co-chaperone is a homodimer that binds a single DnaK molecule. Each monomer consists of a long N-terminal α-helix followed by two antiparallel helices, and a compact β-sheet domain. In the dimer, the long α-helices associate in a non-canonical coiled-coil and the antiparallel helices form a four-helix bundle. Most of the contacts with the DnaK ATPase domain are found in the β-sheet domain.

It has been proposed that the thermal stability of GrpE plays an important role in regulating the activity of DnaK under heat-shock conditions.[20] Reversible unfolding of the long N-terminal α-helices at 50 °C induces a non-Arrhenius temperature dependence of the conversion rate from the high-affinity to the low-affinity states of DnaK,[21,22] which results in a slower chaperone cycle. Reduction of the ADP/ATP exchange activity induces a shift of DnaK population to the high-affinity state under heat-shock conditions, which in turn promotes an increased sequestering of the protein substrate.[23]

We have characterized the effect of *S. cerevisiae* Mge1p stability on its nucleotide exchange stimulation activity. The results shown here demonstrate that a reversible denaturation transition at heat-shock temperatures controls the ability of the co-chaperone to interact with and accelerate the chaperone cycle of mtHsp70. Thus, Mge1p functions as a thermosensor of the mtHsp70 system, although, as it is discussed, through a mechanism different from that described for bacterial GrpE.

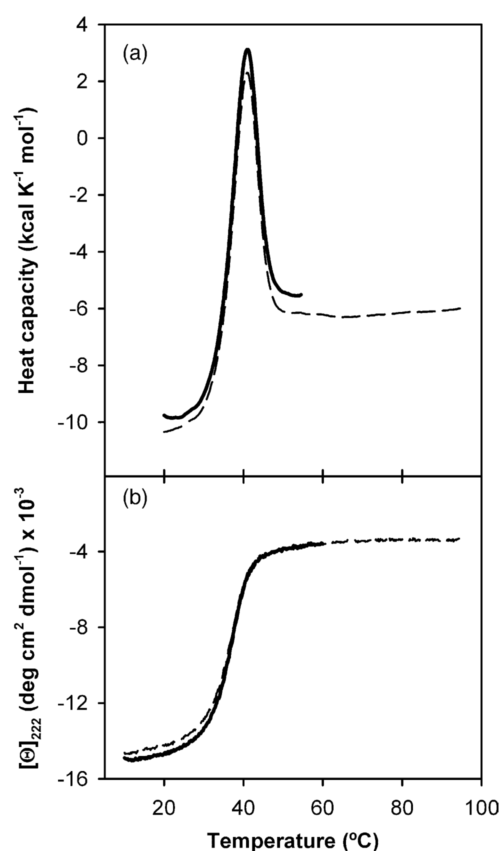

Figure 1. Thermal denaturation of Mge1p followed by DSC and CD. (a) Two consecutive DSC scans of 38 μM Mge1p in 25 mM potassium phosphate (pH 7.0), 100 mM KCl. The sample was first heated to 50 °C (continuous line) and then, after cooling, to 95 °C (broken line). (b) Temperature-dependence of the CD molar ellipticity at 222 nm. A sample containing 30 μM Mge1p in 25 mM potassium phosphate (pH 7.0), was heated from 15 °C to 60 °C (continuous line), cooled and heated again to 95 °C (broken line) in a 2 mm path-length cuvette.

Results

Thermal denaturation of Mge1p is reversible

We have characterized the temperature-induced structural transitions of Mge1 by differential scanning calorimetry (DSC) and circular dichroism (CD) studies. In Figure 1(a), two consecutive DSC scans of a solution of Mge1p in 25 mM potassium phosphate, 100 mM KCl buffer can be seen. In contrast to the bacterial homologue GrpE, which undergoes two well-resolved endotherms with midpoint transition temperatures at 50 °C and 75 °C,[20,21] Mge1p showed only one transition centered at 40 °C. This transition is highly reversible, since reheating of the sample yielded a scan with very similar heat capacity and calorimetric enthalpy values (99% of the area below the curve is recovered). Both transition midpoint temperature and calorimetric enthalpy were largely independent of the concentration of salt in the buffer (not shown).

When the thermal denaturation of Mge1p was characterized by CD, following the molar ellipticity at 222 nm, similar results were found (Figure 1(b)), and 98.7% of the molar ellipticity is recovered after cooling a solution of Mge1p previously heated to 60 °C. It should be mentioned that the molar ellipticity value corresponding to the native state of Mge1p is in the same range as that of GrpE,[20,21] indicating that the α-helical content of both proteins is similar.

Denaturation of Mge1p involves dissociation of the dimer

To better characterize the thermal denaturation of Mge1p, the CD spectra of the protein were recorded

Download English Version:

https://daneshyari.com/en/article/2189871

Download Persian Version:

https://daneshyari.com/article/2189871

Daneshyari.com