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Investigation on different chemical stability of mitochondrial Hsp60 and its precursor

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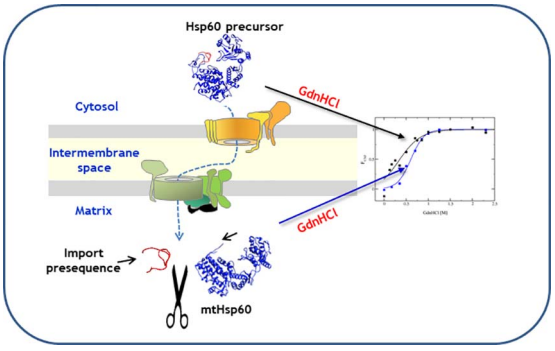
HIGHLIGHTS

- Two different forms of Heat Shock Proteins Hsp60s, mitochondrial and naïve, were studied.
- Circular Dichroism (CD) and Small Angle X-ray Scattering (SAXS) were exploited to compare their oligomeric form and stability.
- Naïve and mitochondrial Hsp60 differ in their native forms.
- Mitochondrial Hsp60 presents more heptamers in solution in respect to naïve form, at equilibrium.
- Mitochondrial and naïve Hsp60 show different stabilities against chemical denaturant.

GRAPHICAL ABSTRACT



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ABSTRACT

In the large class of molecules that maintain protein homeostasis, called molecular chaperones, chaperonins constitute a subclass that specifically assist the correct folding of newly synthesized proteins. Among them, Hsp60 is composed of a double heptameric ring structure with a large central cavity where the unfolded protein binds via hydrophobic interactions and is supported, in this function, by the co-chaperonin Hsp10.

Hsp60 is typically located in the mitochondria, but in some pathological situations, such as cancers and chronic inflammatory diseases, Hsp60 accumulates in the cytoplasm. In these cases, cytoplasmatic Hsp60 is a mixture of mitochondrial Hsp60 secreted from mitochondria upon stress, and its precursor, called naïve Hsp60, never entered into the organella. The difference between the naïve and mitochondrial Hsp60s resides in the absence of the mitochondrial import signal (MIS) in the mitochondrial form, but information on their different structure and stability is still lacking. We present here a study on the stability against a chemical denaturant, of the different cytoplasmic Hsp60 species. By combining Circular Dichroism and Small Angle X-ray Scattering as experimental biophysical techniques to investigate Hsp60, we find that naïve and mitochondrial Hsp60 (mtHsp60) forms differ in their stability. Furthermore, specific responses from the two forms are discussed in terms of the biological environment they are working in, thus opening new questions on their biological function.

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1. Introduction

Stability and function are strictly interconnected in proteins structure and evolution. According to the recently recognized "stability-function trade-off" theory, formulated to explain evolution of new enzymatic specificities, the structural features that attribute stability to proteins appear to be opposed to those that ascribe them activity [1]. In this sense, mutations that are accompanied by a substantial increase of flexibility at expenses of their stability are crucial for the functionality of the proteins [2]. Among the proteins that mostly contribute to cell proteostasis, thanks to their extraordinary functional complexity, are molecular chaperones, and, in particular, belonging to the chaperonins family, the heat shock proteins (HSPs). These proteins play an essential role in many physiological cellular processes, by assisting protein folding, transport and assembly [3,4,5], and in stress conditions they prevent aggregation and promote refolding of damaged proteins [6]. It is therefore not surprising that they are highly involved in a number of severe diseases affecting humans worldwide, such as neurodegenerative diseases and cancer. In fact, HSPs act as strong suppressors of aggregation and toxicity in amyloid diseases [7,8,9] but they are also emerging as therapeutic targets in fighting cancer, as they are strongly co-opted by tumor cells to bind the aberrant proteins and maintain them in a functional conformation [10,11,12]. Among HSPs, Hsp60 lies at the center of the debate for its controversial role in cancer. In fact, although Hsp60 has a mitochondrial localization, in some situations, like cancers or inflammatory pathologies, it accumulates into the cytosol where it is found in a double form: a naïve form that, once produced by the cell nucleus, never enters into mitochondria, and a mitochondrial one, secreted by the organella upon stress [13,14]. The difference between them lies in a presequence of 26 aminoacids (see Fig. 1) constituting the mitochondrial import sequence (MIS) that, useful for transportation in mitochondrial membranes, is cleaved inside them [15]. This distinction provides the protein specific functional properties, in a way that the two Hsp60 forms are oppositely involved in life/death mechanisms [18,19].

In particular, Chandra et al. [20] demonstrated that in some apoptotic systems, mitochondrial Hsp60 (mtHsp60) directly interacts with procaspase-3 in cytosol enhancing caspase-3 maturation and activation in a "pro death" function. In other systems that involve mainly naïve Hsp60 accumulation in cytosol, a classical chaperone "pro survival" role is supposed, since the protein knockdown is observed to favour cell death. In contrast to what happens for mtHsp60 case, in this case the peculiarity of Hsp60 bonding with procapsase-3 would embed protein activation inhibiting the apoptotic trigger. Therefore, mitochondrial protein actives pro apoptosis mechanisms, whereas naïve Hsp60 has been proposed as survival protein with important implications in cancer cells destine [20].

Despite the great interest in this protein, mtHsp60 structure and stability have not been widely studied, probably because of its close resemblance to the very well-known bacterial homolog GroEL. Just recently mtHsp60 crystallographic structure in complex with its co-chaperonin Hsp10 was obtained [21]. Starting from GroEL, we previously characterized oligomeric equilibria of naïve Hsp60 and we found that, differently from its bacterial homolog only organized in a tetradecameric conformation made up of two heptameric rings, naïve Hsp60 seems to exists in a dynamic heptamer/tetradecamer equilibrium. Moreover, we demonstrated that naïve Hsp60 is much less chemically stable in respect to GroEL [22,23]. However, apart from these studies on Hsp60/GroEL comparison, to our knowledge, the two Hsp60 forms have never been directly compared in their stability properties. Here we present a study of the effect of Guanidine Hydrochloride in solution to provide information on mtHsp60 stability and compare it with data on its precursor naïve Hsp60.

Small Angle X-ray Scattering (SAXS) experiments performed with Synchrotron Radiation showed different stabilities of naïve and mtHsp60 in both oligomeric dissociation from tetradecamer to heptamer and then to monomer, and in the unfolding stages. Circular dichroism (CD) measurements have provided information on the change in the secondary structure due to protein unfolding, confirming that mtHsp60 has less stability and lower cooperativity in the chemical denaturation transition with respect to its precursor. Finally, the stabilizing effect of the MIS tail has been evaluated by means of a Predictor of Naturally Disordered Regions algorithm.

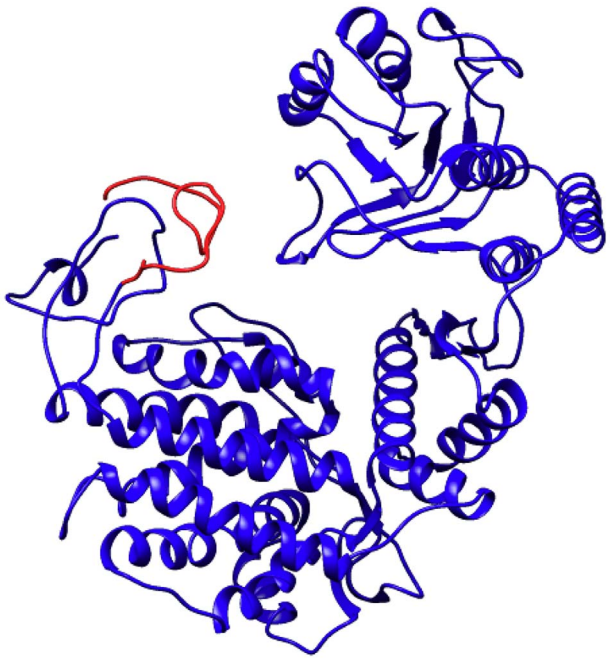

Fig. 1. Naïve Hsp60: in red the MIS sequence is highlighted (MLRLPTVFRQ MRPVSRVLAP HLTRAY). The image is rendered using the structure from [16] and the UCSF Chimera package [17]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Sample preparation

Mitochondrial and naïve recombinant Hsp60 were obtained from ATGen (Seongnam, South Korea) in stock solution at 8.3 μM (0.5 *mg/mL*) (buffer 20 *mM* Tris pH 8.0, 100 *mM* NaCl and 10% glycerol (*w*/w)) and 16.6 μM (1 *mg/mL*) (buffer 20 *mM* Tris pH 8.0 and 10% glycerol (*w*/w)) respectively. We diluted both the proteins from stock solutions to reach the concentration of 2.7 μM. Hence, protein solutions were filtered through a series 0.22 μm membrane and 1*MDa* Vivaspin filters with Polyethersulfone membrane (Sartorius, Germany). Higher protein concentrations were obtained by using Vivaspin concentrators with Polyethersulfone membrane and 10 *kDa* molecular weight cut-offs (Sartorius, Germany). For Circular Dichroism and Small Angle X-ray Scattering experiments protein concentration was checked by measuring the area under the corresponding chromatographic absorbance peak (280 *nm*) [22].

Guanidine Hydrochloride (GdnHCl) was bought from SIGMA (St. Louis, MO, USA) and a stock solution of 6 *M* GdnHCl was prepared for subsequent dilutions. GdnHCl concentration was finely controlled by measuring the refractive index of the 6 *M* GdnHCl mother solution [24]. GdnHCl stock solution was then diluted to the needed concentration according to the protein sample studied for denaturation experiments. Each denaturation point corresponds to a separate solution which was prepared incubating denaturant and protein until equilibrium was reached at 20°C.