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The C-terminal α-helices of mammalian Hsc70 play a critical role in the stabilization of α-synuclein binding and inhibition of aggregation

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ABSTRACT

Protein misfolding, followed by aggregation and amyloid formation is an underlying pathological hallmark in a number of prevalent diseases, including Parkinson's (PD), Alzheimer's (AD) and Type 2 diabetes (T2D). In the case of PD, the aggregation of α-synuclein protein (α-syn) has been shown to be highly cytotoxic and to play a key role in the death of dopaminergic cells. Thus, inhibition of the aggregation process may be considered as an attractive avenue for therapeutic intervention. In this respect, molecular chaperones, known to promote proper folding of proteins, are able to inhibit protein aggregation thus preventing amyloid formation. In this work, the effect of the constitutively expressed chaperone Hsc70 and its various domains on α-syn aggregation have been investigated using different approaches. The results show that the C-terminal domain alone (residues 386–646) is as efficient in inhibiting α-syn aggregation as the entire Hsc70 protein, by increasing the lag phase for α-syn oligomeric nucleus formation, suggesting that the chaperone interacts with and stabilizes α-syn monomers and/or small aggregates. Deletion of the C-terminal helices (residues 510–646), which are known to play the role of a lid locking target peptide ligands in the peptide-binding site of the chaperone, strongly reduced the efficiency of inhibition of α-syn aggregation indicating that these helices play an essential in stabilizing the interaction between Hsc70 and α-syn. Furthermore, the effects of Hsc70 and its structural domains on aggregation appear to correlate with those on cytotoxicity, by reducing the fraction of α-syn toxic species to various degrees. Together these results suggest a mechanism in which inhibition of synuclein aggregation is the result of monomeric synuclein binding to the chaperone as any monomeric target unfolded protein or peptide binding to the chaperone.

1. Introduction

A common theme shared by Parkinson's disease (PD) and many other disorders is the abnormal folding or clearance of potentially cytotoxic protein species. In the case of PD, the property of aggregation is attributed to the α-synuclein protein (α-syn) [1,2]. The cascade of α-syn is believed to progress from the protein misfolding, to the formation of oligomers, the maturation of protofibrils and finally the formation of mature fibrils [2]. This pathological conversion of misfolded proteins to amyloid aggregates can form cytotoxic species [2].

It is well established that molecular chaperones play an important role in preventing protein misfolding and aggregation [3–5]. Chaperones and particularly the 70 kDa Heat Shock Protein Family (Hsp70s) have thus emerged as key modulators of protein amyloidogenesis [6,7], and several studies demonstrated their role in inhibiting the fibrillogenesis of different amyloidogenic proteins such as α-syn [8–10] and β-amyloid [11]. Other studies indicated that Hsp70 protect against α-syn toxicity in vitro [8,11] and reduce the amount of α-syn aggregates in vivo [8].

At the mechanistic level, Hsp70s exhibits two activities, an ATP-independent "holding" activity that consists in a mere, high affinity, binding to an unfolded protein thus preventing it from aggregation, and an ATP- and co-chaperones-dependent "folding" activity

Abbreviations: α-syn, alpha synuclein; Hsp70, heat shock protein 70; PD, Parkinson disease; ThT, thioflavine T; DLS, dynamic light scattering; R_H, hydrodynamic radius; AFM, atomic force microscopy; PBSD, peptide binding sub domain.

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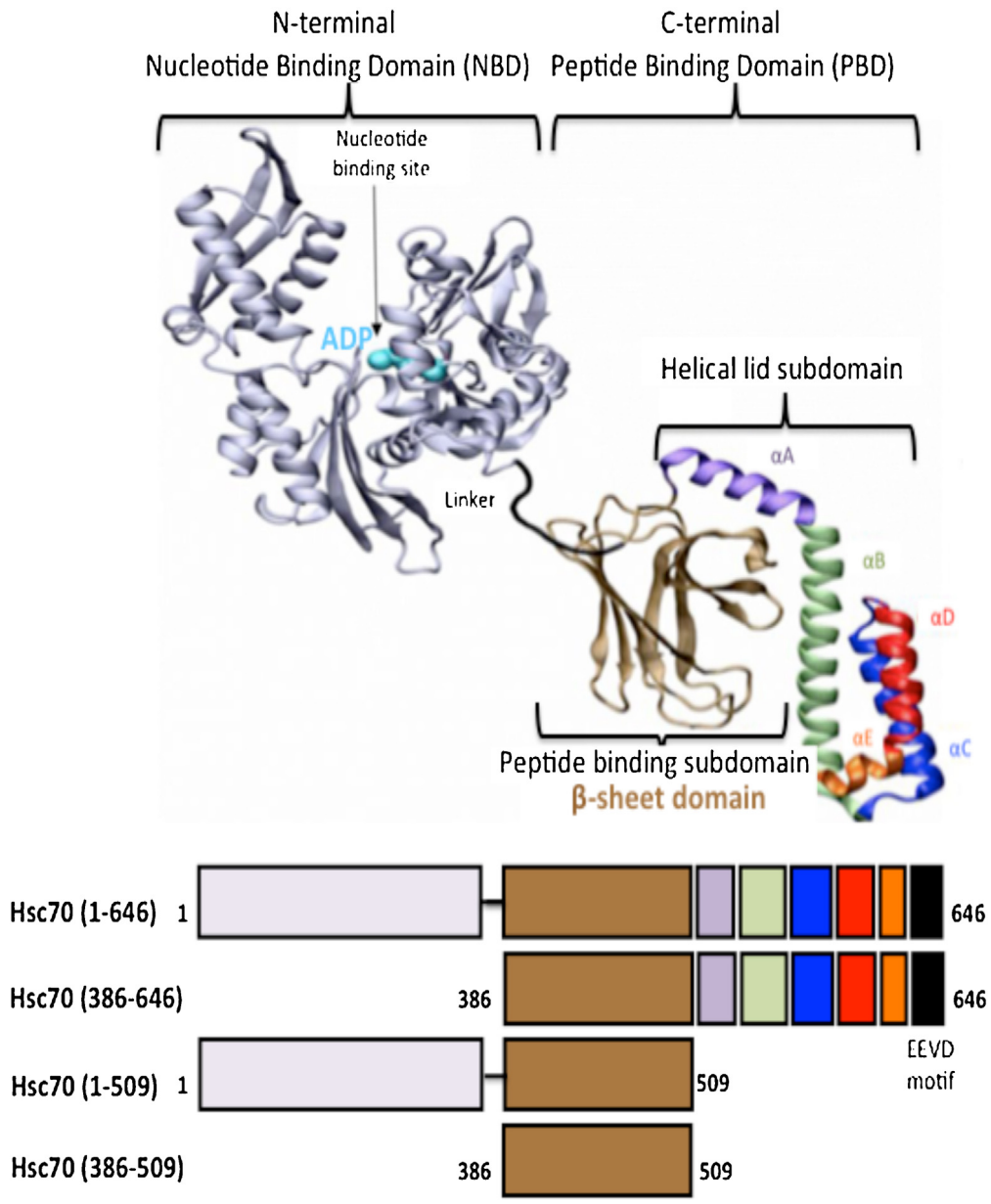

Fig. 1. *Representation of Hsc70 and its respective structural domains.* Top: Structure of DnaK based on the PDB file 2KHO [37] 19. Bottom: Schematic representation of Hsc70 and its structural domains represented by different colors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that results in the efficient folding, by coupling repeated cycles of ATP binding and release to cycles of association and dissociation to the misfolded protein [12–14]. The constitutive member of the Hsp70s family is made of two structural and functional domains [7,15–17]: an N-terminal ATPase domain, known also as nucleotide binding domain, NBD (residues 1–385) [18] and a C-terminal peptide-binding domain (PBD: residues 386–646) involved in unfolded protein binding [19,20] (Fig. 1). The C-terminal domain is in turn composed of 2 subdomains: the peptide-binding subdomain (SBSD) per se (residues 386–509) and the C-terminal α-helical subdomain (residues 510–646) that plays the role of a lid (composed of 5 helices) that regulates substrate binding and release under the control of ATP binding and hydrolysis [19,20].

In this work, the effect of the constitutively expressed chaperone Hsc70 and its various domains on α-syn aggregation have been investigated using different approaches.

2. Material and methods

2.1. Expression and purification of α-syn

Recombinant wild type α-syn was overexpressed in *E. coli* BL21 using a pET-28a vector coded with a T7 promoter and was purified as described in [21]. α-syn concentration was determined using an extinction coefficient of 5960 M^{-1}/cm at 280 nm. Before using, pure lyophilized α-syn was resuspended in 50 mM Tris–HCl (pH 7.4), 100 mM KCl buffer and was filtered through 0.22 μM pore size filter.