STRUCTURE STUDY OF SMALL HEAT SHOCK PROTEIN 27

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Dedication

To my family

STUCTURE STUDY OF SMALL HEAT SHOCK PROTEIN 27

by

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THESIS

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Abstract

Molecular chaperones are a class of oligomeric proteins that play a critical role in preventing the aggregation of non-native protein so that these proteins can later be refolded. Chaperones are ubiquitously expressed in all the kingdoms of life where their function is to counteract cellular stress and to maintain protein homeostasis. One subgroup of molecular chaperones is characterized by low molecular weight and are termed small heat shock proteins. The focus of the proposed research is the small heat shock protein 27 (Hsp27). Hsp27 is an ATP independent chaperone that is overexpressed in response to heat shock, radiation damage, oxidative damage, or other cellular stress in order to preserve protein homeostasis. Notably, there is an oligomeric reshuffling of Hsp27 from large oligomers to functional dimers under stressful conditions. Phosphorylation at three serine residues, 15, 78, 82 initiate the dynamic equilibrium change from large oligomeric complexes to much smaller dimeric subunits.

Accumulation of misfolded protein causes numerous diseases that impacts the daily life of millions of individuals. For example, the misfolding or aggregation of microtubule associated protein Tau, and neuron associated alpha-synuclein are the pathological hallmarks for Alzheimer's and Parkinson disease, respectively. Studies have suggested a significant role of Hsp27 in inhibiting the aggregation of both Tau and alpha-synuclein. The contact area between Hsp27 and its client proteins Tau and alpha-synuclein has been characterized by a variety of biophysical techniques that enhance our understanding neurotherapeutics. However, a high-resolution atomic structure of the monomeric full length Hsp27 still remains ambiguous because of the struggles in obtaining a homogeneous purified product for reconstruction and by the fact that the protein has intrinsic disorder. This intrinsic disorder is a functional attribute of a large portion of the N-terminal and C-terminal regions. This project aims to apply cryo-EM 3D

reconstruction techniques to elucidate a high-resolution structure of human Hsp27 in complex with its substrates Tau and alpha-synuclein with the idea that the association with substrate will stabilize previously disordered regions in Hsp27.

Table of Contents

List of Figures

Chapter 1: Protein Folding and Small Heat Shock Protein 27

1.1 Protein Folding and Molecular Chaperone

Proteins are one of the most sophisticated biological macromolecules that play a vital role in all cellular functions.[1] In general, proteins must be folded into a proper three-dimensional conformation to attain their biological function.[2] The protein folding process was initially described by Christian Anfinsen denatured ribonuclease refolding experiment. The experiment demonstrated that the denatured ribonuclease was able to independently refold to its native conformation without the participation of other enzymes. [3] Interestingly, protein must maintain a certain degree of structural flexibility in order to interact with numerous substrates. Majority of protein are barely thermodynamically stable under phycological cellular condition.[4] Diverse physiological stressful conditions such as heat and oxidative stress can drastically disrupt protein intermolecular interactions, which consequently lead to loss of protein function.[5] Not surprisingly, misfolding or aggregation of proteins causes detrimental effects in cellular homeostasis that will eventually lead to the progression of various diseases such as type 2 diabetes, and cardiovascular disease.[6] A class of proteins, termed molecular chaperones, usually serve as the cell first line of defense during stressful conditions by interacting with non-native protein to maintain proteostasis to acquire its native conformation. [7] Chaperones can be categorized based on their molecular weight such as heat shock protein 100, heat shock protein 60, heat shock protein 70 as well as small heat shock proteins like Hsp27. These proteins are widely involved in nascent peptide folding, partially denatured protein refolding and prevent protein irreversible aggregation. Both Hsp60 and Hsp70 are able to refold the non-native protein in ATP dependent folding cycle. Hsp70 primarily refolds the nascent synthesized proteins. While

Hsp60 targets for refolding the late stage of protein which is not able to reach thermodynamic favorable state.[8]

1.2 Small Heat Shock Protein 27

Small heat shock proteins are ubiquitously expressed in all kingdoms of life and play essential roles in cellular stress response in an ATP independent manner. Humans express 10 different small heat shock proteins that are categorized by their molecule weight.[9] A small heat shock protein's main function is to preserve a misfolded protein in a folding competent state. The misfolded protein can then be transferred to a chaperonin such Hsp60 and its co-chaperonin Hsp10 to be refolded to its native conformation through an ATP dependent hsp60/10 mechanism or Hsp70 protein [10] As the name implies, small heat shock protein 27 is 27 kDa in size and is encoded by the HSBP1 gene. Ironically, Hsp27 is characterized as an intrinsically disordered protein due to its unique architecture where an extremely disordered long amino terminal region and a short flexible carboxyl terminal region flank a central α-crystallin domain (ACD) that is highly conserved in the small heat shock protein family.[11] Hsp27 can form a dimeric complex that is considered the fundamental functional building block that is used to form larger oligomers. The dimer interaction is through hydrogen bonding between monomers to form a beta sheet in the central α-crystallin domain.[12]

The central α-crystalline domain from residues 86-169 is composed of nine beta stands that form two anti-parallel β-sandwich folds which the beta sheet interaction generated between two of these ACD domains modulates the dimerization of small Hsp27. The dimer can then assemble to form an oligomer of up to 900 kDa via interactions across dimers to adjacent dimers using both the amino and carboxy terminal regions which then bind to the ACD domain. [13] Similar to other mammalian small heat shock proteins, Hsp27 can assemble to oligomers of

various sizes that rapidly dissociate to small oligomers and dimers under stress induced phosphorylation.[14]

Protein phosphorylation is evaluated as a quintessential post translational modification in eukaryotic cells. Reversible phosphorylation plays a key role in signal transduction pathways where it serves as a mediator for cells to rapidly respond to both intracellular and intercellular signals.[15] For Hsp27, phosphorylation plays a role in regulating the dynamic equilibrium shift from large oligomeric complexes to dimers and back again. Serine 15 (S15), Serine 78 (S78) and Serine 82 (S82) located in the amino terminus and Threonine 43 (T 143) in the central α-crystallin domain are characterized as the phosphorylation sites in Hsp27 responsible for oligomer disruption. Preliminary research has illustrated that the phosphorylation at Serine (S15), Serine (S78) and Serine (S82) are primarily inducing conformational changes from large oligomeric complexes to dimers.[16] Numerous of kinase are able to phosphorylate Hsp27 such as mitogen actived protein kinase activated protein kinase 2, 3 ,5 (MAPKAPK2, 3, 5). Serval research have demonstrated protein phosphatase 2A (PP2A) is able to be dephosphorylated Hsp27 to reform large oligomer complex.[17]

The disordered N-terminal region of Hsp27 is composed of the first 93 amino acids which are predominantly hydrophobic or aromatic amino acid. The WF/EPF motif from residue 16 to 19 has been shown to play a critical role in Hsp27 protein chaperoning activity. The highly conserved N-terminal region has been shown to stabilize high molecular weight oligomers.[18] However, the intrinsic disorder found in the N-terminal region is the primary obstacle for high resolution structure determination. The N-terminal region has been found to bind to non-native substrate protein to achieve its chaperone function. In the non-native conformation, the hydrophobic surface of the substrate protein will be exposed to the aqueous environment. The N-

terminal region of Hsp27 has a tendency to bind to partially denatured protein through the hydrophobic residues found on its surface.[19] Even though partially denatured proteins can bind to the ACD domain, binding the N-terminal region is required to maintaining its chaperone function.[20] The highly conserved ACD domain forms an immunoglobulin like structure that is composed of two β sheets. Beta strands 4, 5 and extended strands 6+7 form one beta-sheet. Another beta sheet is composed of strands 3, 8 and 9. Beta strands 4 and 8 form a hydrophobic groove which is versatile region in charge of substrate binding.

The C terminal region, which contains the last 36 residues, is composed of polar and charged residues. The higher flexibility of this region contributes to the intrinsic disordered characteristics of Hsp27 as well. The IXI/V motif can interact with the β 4 and β 8 groove to facilitate large oligomer formation.[21] The protein interaction between the IXI/V motif and β 4/ β 8 is one of the best characterized protein-protein interactions primarily in charge of oligomer stabilization. This protein-protein interaction drives the association of the flexible IXI motif with the groove between β 4 and β sheet 8.[22]

1.3 Substrate Neuronal Protein Tau

Alzheimer disease (AD) is a progressive neurodegenerative disease central nervous system that predominantly affects individuals over 65. Currently, more than 6 million Americans are afflicted from Alzheimer disease. The clinical manifestations include memory loss, cognitive impairment, and disorientation. Despite significant research effort in therapeutic treatment of Alzheimer, it remains an incurable condition that impacts a large population. Preliminary investigation has revealed that the formation of amyloid plaques from beta-amyloid peptide and

neurofibrillary tangles consisting of aberrant hyperphosphorylated microtubule-associated protein tau (Tau) are the pathological hallmarks of Alzheimer disease.[23]

Microtubule-associated protein Tau, which is primarily expressed in neurons and encoded by the MAPT gene, plays a crucial role in stabilizing the microtubules that are integral components of the cytoskeleton, responsible for intracellular transport.[24] However, the abnormal behavior of Tau has been shown to be similar in various neurodegenerative disorders, including Alzheimer's disease. In these disorders, Tauopathy is characterized by the accumulation of insoluble amyloid filaments containing Tau protein, resulting in irreversible damage to neuronal cells.[25]

Human MAPT gene is able to encode 6 isoforms from 352 to 441 amino acids contributed from RNA alternative splicing. [26] The tau can be characterized in three domains which are N terminal projection domain, proline rich domain and microtubule binding domain. The interaction between Tau and microtubule is dynamically modulated by serval post translational modifications including phosphorylation, glycosylation, and nitration.[27] Tau aggregation is associated with neurodegenerative disorders and is directly correlated with Tau hyperphosphorylation. The normal level phosphorylation of Tau occurred at serval serine or threonine residues and mediates the dynamic binding equilibrium between Tau and microtubular.[28] The normal phosphorylation occurs at different sites for Tau isomers. Hyperphosphorylation leads Tau incompetent to bind to the microtubular, causing the destabilization of microtubular leading the neuron death. The hyperphosphorylated Tau thermodynamically favors the formation of neurofibrillary tangles (NFTS), which has been identified in Alzheimer disease patient.[29] The high atomic resolution of Tau from Alzheimer disease brain sample, determined by cryo-EM, enhances the current understanding of tauopathy.

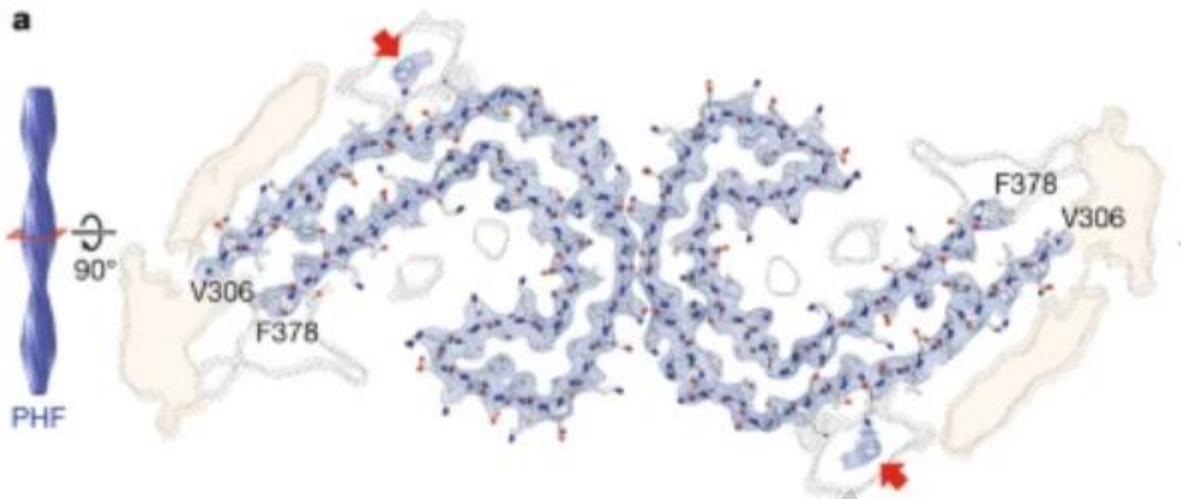

Figure 1.1 Cryo-EM atomic model of Tau filament from Alzheimer's patient brain [30]

Recent studies have shown that Hsp27, a small heat shock protein, is competent to inhibit Tau aggregation both in vitro and in vivo. Hsp27 has been shown to interact with the Tau PHF domain, which is responsible for filament formation, and prevent the aggregation of Tau proteins. Moreover, studies on the truncation of the N-terminus of Hsp27 have indicated that the central ACD alone is insufficient to inhibit Tau filament formation. The N-terminus of Hsp27 has been shown to play a vital role in its chaperone activity, as it is responsible for the oligomerization of Hsp27 and its subsequent interaction with abnormal proteins. This demonstrates the significance of understanding the molecular mechanisms by which Hsp27 interacts with Tau and other client proteins, in order to advance potent therapeutic interventions. [31]

1.4 Substrate Neuronal Protein Alpha Synuclein

Parkinson disease (PD), a central nervous system neurodegenerative disorder is caused by substantial loss of dopaminergic neurons. Over 95% of cases occurs the people over 55. The

clinical symptoms of Parkinson disease are typically characterized by rigidity, hypophonia and drooling.[32] The primary pathological hallmark of Parkinson disease is Lewy bodies which is composed of various of filament proteins such as alpha synuclein.[33] The toxic alpha synuclein filament is able to disrupt the cell membrane which is able to lead the death of neuron cells. Human alpha synuclein encoded from SNCA gene is a small 14.5 kDa presynaptic protein composed of 140 amino acids. Alpha synuclein can be characterized in three domains. The N terminus (1 to 60 residues) which contains lipid binding motif to interact with neuron cell membrane; the center hydrophobic domain known as NAC and the negative charge, disordered short C terminus.[34] While α-synuclein has been studied extensively over the past few decades, the clear physiological function of α-synuclein in regulating central neurons remains enigmatic.

Alpha synuclein is most abundantly localized in axon terminal in neurons, which plays vital role in modulation of synaptic vesicle transportation.[35] The association between alpha synuclein and synaptic vesicle is mediated by calcium ion binding to C terminus residues. The N terminal membrane binding domain of alpha synuclein is able to bind to phospholipid bilayers of synaptic terminal cell membrane. Preliminary C terminal truncation experiment demonstrates that highly negative charged C terminus plays critical role in association with synaptic receptor protein (SNARE) to facilitate vesicle fusion. [36]