ABSTRACT

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Title of Ph.D. Thesis: Study the Molecular Basis of Interaction of Heat Shock Factor 1 with its Activator Azadiradione

An evolutionarily conserved transcriptional program known as Heat Shock Response (HSR), also called Proteotoxic Stress Response (PSR) maintains protein homeostasis in a healthy cell. Heat Shock Factor 1 (HSF1), a transcription activator is the master regulator of HSR. Under normal cellular conditions, HSF1 stays as an inactive monomer. Upon exposure to stress, HSF1 undergoes homotrimerization/ homo-oligomerization and binds with its recognition sequence called Heat Shock Element (HSE) present in the promoters of its target genes. These genes encode proteins, such as inducible molecular chaperones and proteases that are involved in the refolding and/or clearance of misfolded/aggregated cellular proteins, thereby restoring protein homeostasis. Individuals suffering from neurodegenerative diseases and some elderly people have a compromised HSR (HSF1 activity), which necessitates the development of small molecules that can forcibly activate HSF1 to upregulate the maintenance of cellular protein homeostasis. Cancer cells, however, maintain HSF1 in a constitutively active state to support their proliferation, survival, invasion, and metastasis. Therefore, inhibition of HSF1 in cancer cells could be a useful strategy to combat the disease. Unlike any other HSF1 activator reported so far, Azadiradione (AZD), a triterpenoid isolated from *Azadirachta indica* (Neem) seeds, was shown to stimulate mammalian HSF1 activity by direct interaction in the absence of stress, at a concentration non-toxic for cells. AZD has been reported to successfully restore protein homeostasis (quality control) in fruit fly and mouse models of neurodegenerative diseases. The present study aimed at understanding the mechanism of AZD-induced activation of human HSF1 by *in vitro* studies. The interaction of wild-type (WT) human HSF1 and its derivatives (which includes HSF1 devoid of certain structural domains as well as a mutant form of HSF1) with AZD was studied employing various biochemical and biophysical methods. This study revealed that AZD induces HSF1-HSE binding by facilitating homo-oligomerization of monomeric HSF1, mediated by the interaction of AZD with the protein's DNA Binding Domain (DBD). Surprisingly, and in stark contrast to stress-induced HSF1 activation, AZD-induced activation of HSF1 is independent of its oligomerization domain. AZD-induced multimerization of HSF1 does not require the presence of HSE (HSE-DNA). Interestingly, AZD inhibits the binding of the pre-assembled trimers/ oligomers of WT-HSF1, the constitutively trimeric/oligomeric form of HSF1, and the transactivation domain deletion mutant of HSF1 (HSF1-ΔTAD) with HSE-DNA. AZD mediates this effect by promoting the formation of amyloid-like aggregates of the HSF1 constitutive trimer/ oligomer and HSF1-ΔTAD. However, the HSF1-WT trimer/ oligomer does not form aggregates upon AZD exposure. Our results further suggest that the interaction of AZD with HSE-DNA also contributes to HSF1-HSE binding. We also analyzed our results in light of published X-ray crystal structures of HSE in complex with HSF1-DBD to understand possible interaction sites of AZD on the HSF1-HSE complex. Our results are consistent with the idea that AZD facilitates the intermolecular interaction of HSF1-DBD subunits either directly or indirectly through their winged helix-turn-helix motifs, thereby contributing to the enhancement of HSF1-HSE binding affinity.

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