Localisation of the molecular chaperone site of 14-3-3ζ; an intracellular protein associated with toxic neurological protein aggregates

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Abstract

14-3-3 proteins are a family of acidic, dimeric, phospho-serine binding proteins, which are ubiquitously expressed in all mammals. There are 7 known isoforms in mammals (β, γ, ε, ζ, η, τ, σ), which have similar structures and roles. 14-3-3 proteins interact with over 200 target proteins and regulate many roles including apoptosis, protein transportation, mitosis and signal transduction. Due to these diverse roles, 14-3-3 proteins are associated with many diseases, e.g. cancer and neurodegenerative diseases. 14-3-3 is co-located with many neurological protein aggregates; however the role of 14-3-3 in these diseases is unknown. Recently the molecular chaperone action of 14-3-3ζ was described whereby 14-3-3ζ is able to interact with and stabilise aggregating target proteins.

Previous investigations into the regions responsible for chaperone action showed that the C-terminal extension and the polar face of the amphipathic binding groove of 14-3-3ζ are unlikely to be involved in chaperone action. Here, the investigation into the site and mechanism of the molecular chaperone action is extended to target two major hydrophobic regions of 14-3-3ζ: the hydrophobic face of the amphipathic binding groove and the dimer interface.

The hydrophobic face of the amphipathic binding groove is not a critical region for the chaperone action of 14-3-3ζ. This was determined by the mutations of exposed hydrophobic residues, V176, L216, L220 and L227 and assessing the chaperone ability of these proteins compared with WT 14-3-3ζ proteins against the amorphous aggregation of alcohol dehydrogenase and reduced insulin.

The dimer interface was determined to be involved in the chaperone activity of 14-3-3ζ. This region was investigated by targeting hypothesised salt bridging sites in the dimer interface (D21 and E89). Disruption of this region can also be achieved via phosphorylation of S58. The 14-3-3ζ protein, S58D, is a phospho-mimic which exhibits a similar dimer disruption. The disruption caused by these mutations was assessed and the chaperone ability was tested

against amorphous aggregation of alcohol dehydrogenase and reduced insulin and compared to WT 14-3-3ζ. These dimer disrupted proteins exhibited enhanced chaperone ability, implying exposure of the dimer interface is important for the chaperone action of 14-3-3ζ. In addition these 14-3-3ζ mutants also exhibited a shift in the monomer-dimer equilibrium which results in the increased production of monomeric 14-3-3ζ. This shift in the monomer-dimer equilibrium correlates with the enhanced chaperone ability of 14-3-3ζ, suggestive of the 14-3-3ζ monomer being an important chaperone active unit.

To further the investigation of the role of the dimer interface in the chaperone action of 14-3-3ζ the interaction with a physiological lipid mimic was undertaken. The physiological lipid, sphingosine, is known to interact with 14-3-3 and cause disruption to the dimer interface in order to allow phosphorylation of S58. The interaction with a sphingosine mimic caused disruption to the dimer, and the chaperone ability in the presence of this mimic was assessed. There no observed effect on the chaperone activity of 14-3-3ζ as a result of this interaction. However the disruption caused by this interaction is minor and may not be sufficient to cause enough disruption to expose significant region of the dimer interface required for enhanced chaperone ability.

Small angle scattering studies confirmed that the dimer interface is involved in the chaperone action of 14-3-3ζ. Modelling the interaction between aggregating ADH and 14-3-3ζ revealed that ADH interacts with dimeric 14-3-3ζ via a region of the dimer interface. This allows the formation of the dimeric structure which maintains the stability of the ADH-14-3-3 complex. The independent movement of the two interaction regions of the dimer interface makes it possible for one side of the dimer interface to dissociate allowing the interaction with the aggregating target protein. The interactions on the other side of the dimer interface remain intact allowing the dimer to be maintained. This maintains the stability that comes with the dimeric form of 14-3-3ζ whilst still allowing the interaction with aggregating protein via the hydrophobic dimer interface.

This investigation of the chaperone activity of 14-3-3ζ has revealed that 14-3-3 acts as a molecular chaperone via the dimer interface. This interaction occurs when half of the dimer interface dissociates to allow access to amorphously aggregating target proteins. This allows the other side of the dimer interface to maintain salt bridging interactions and retain the dimeric state of the 14-3-3ζ protein with the associated stability. This investigation is the first

instance in which a chaperone protein has been modelled interacting with an aggregating target protein. It also opens up the role of the dimer interface in 14-3-3 function, and clarifies that the monomeric 14-3-3 unit is unlikely to have a role in 14-3-3 pathology due to its inherent reduced stability.

This thesis has provided more in depth knowledge about the chaperone capability of 14-3-3ζ and the potential role of 14-3-3ζ in neurodegenerative disease. The precise function of 14-3-3 proteins in these diseases is not well understood, with 14-3-3 acting as both positive and negative regulator of protein aggregation. The determination of the mechanism of chaperone function of 14-3-3ζ provides important information which can be utilised to further investigate the role of 14-3-3 in these diseases, leading to the development of new therapeutic techniques to overcome these debilitating diseases.

Declaration

Part of the written work in Chapter 1, was written as part of my honours year at the University of Adelaide. With the exception of this, I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Date:

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"We are so often caught up in our destination that we forget to appreciate the journey, especially the goodness of the people we meet along the way…."

~Unknown

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