THE SHAPE AND STABILITY OF THE GTPASE DOMAIN FROM HUMAN SEPT4/BRADEION-β AND ITS INTERMEDIATE ON CHEMICAL UNFOLDING

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The septins are a family of conserved proteins involved in cytokinesis and cortical organization. An increasing amount of data implicates different septins in diverse pathological conditions including neurodegenerative disorders, neoplasia and infections. Human SEPT4 is a member of this family and its tissue-specific ectopic expression profile in colorectal and urologic cancer. Thermal unfolding of the GTPase domain of SEPT4 (SEPT4-G) revealed an intermediate which rapidly aggregates into amyloid-like fibers under physiological conditions. Here, the combined use of fluorescence spectroscopy, circular dichroism, right-angle light scattering and SAXS allowed these analyses to be extended to the chemical unfolding and stability studies of SEPT4-G. Urea induced unfolding of SEPT4-G proceeds via the formation of an intermediate state, stable in approximately 1 M urea, which unfolds further at higher urea concentrations. The intermediate is a compact dimer which is more spherical than the native structure but is unable to bind GTP. It has an increased hydrophobic surface and a reduced α-helical secondary structure. At 1 M urea concentration, the intermediate state was plagued by irreversible aggregation at temperatures above 30ºC. However, higher urea concentration resulted in a marked decay of the aggregation, indicating that the intermediate structures may be necessary for the formation of these aggregates. Biophysical studies of septin stability may provide important insights into the understanding of their roles in important physiological and pathological processes.

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