KINETIC ANALYSIS OF AGGREGATION OF THE GTPASE DOMAIN OF HUMAN SEPT4

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Septins are an evolutionary conserved family of GTP-binding and filaments-forming proteins. In mammals they are involved in a variety of cellular processes such as cytokinesis, exocytosis, and vesicle trafficking. The sequences of all members can be divided into three domains: a variable N-terminal domain, a GTPase domain, and a C-terminal region which generally includes sequences characteristic of coiled-coils. The *SEPT4* gene is physiologically expressed mainly in normal cerebral tissue, interestingly however, under certain pathological conditions, human SEPT4 is also expressed in some types of cancer. Furthermore, SEPT4 has been reported to accumulate in tau-based filamentous deposits and cytoplasmic inclusions in Alzheimer's and Parkinson's diseases respectively. Thermal unfolding of the GTPase domain of SEPT4 (SEPT4-G) revealed an unfolding intermediate which rapidly aggregates into amyloid-like fibers under physiological conditions. In this study, the kinetic analysis of aggregation of SEPT4-G was monitored using extrinsic fluorescence and circular dichroism spectroscopy. The aggregates have the ability to bind specific dyes such as Congo red and Thioflavin-T (ThT), suggesting them to be amyloid in nature. Fibrils formation was monitored by the increase in ThT emmission and electron microscopy. The structural stability was studies as a function of temperature, pH, metal ions and protein concentration. 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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