THE ROLE OF GTP BINDING IN SEPT6 BEHAVIOR AND ITS HYDROLYTIC ACTIVITY

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Human septin 6 (sept6) is expressed widely and ubiquitously in human tissue, its highest levels of expression are observed in lymphoid and haematopoietic tissues. Its improper assembly can lead to pathological states. Filaments and rings of septins have been observed both *in vivo* and *in vitro***.** Studies suggest a role of SUMOylation, phosphorylation and dephosphorylation, GTP binding and hydrolysis in complex formation and regulation. In this work we investigate the role of GTP binding and hydrolysis in sept6 behavior. The predicted amino acid sequences of sept6 contain a consensus sequence for GTP binding. To test if sept6 is able to bind and hydrolyze GTP, protein purified without GTP was incubated with 2 mM GTP. The GTP content was analyzed by HPLC ion exchange. We find that sept6 is able to bind GTP but does not hydrolyze it. Using circular dichroism and fluorescence spectroscopy, we observed differences in sept6's secondary and tertiary structures depending on if it was purified with GTP bound or not. Thermal unfolding shows that sept6 purified in a buffer without GTP is more susceptible to temperature than septin purified with GTP, presenting a one state transition profile with midpoint transition temperature (T_m) around 37ºC, while sept6 with GTP bounded showed a T_m around 50ºC. In summary, we have observed that sept6 is able to bind but not hydrolyze GTP, and structural differences seem to be linked to the presence or not of GTP in the binding site. This structural change results in different temperature susceptibility. We are performing some experiments using electronic microscopy to obtain more structural data that can help elucidate the role of GTP binding by sept6.