ORGANIZATION AND STABILITY OF THE SEPTIN HETEROFILAMENT IS MEDIATED BY COILED-COIL FORMATION

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Septins comprise a conserved protein family that binds guanine nucleotide and forms heterofilaments. In structural terms, they have a common organization: a central GTPase domain, an N-terminal domain and a C-terminal domain, the latter predicted to form coiled coil structures. Currently, the best-characterized human septin heterocomplex (SEPT2/SEPT6/SEPT7) reveals the importance of the GTPase domain in filament assembly. However, the absence of electron density for the C-terminal domains makes its function still unknown. Studies with mammalian septins, and those from other organisms such as Caenorhabditis elegans and Saccharomyces cerevisiae suggests that some groups of septins (e.g. II and IV in mammals) interact via their C-terminal domains and this could be fundamental for correct filament assembly. As such, this work aimed to study the homo/heterotypical affinity for the C-terminal domains of human septins belonging to groups II (SEPT6C/8C/10C/11C) and IV (SEPT7C), investigating whether this domain contributes to the preference for heterotypic interactions during the assembly of the heterofilament. The C-terminal domain interactions were investigated using analytical ultracentrifugation and circular dichroism spectropolarimetry. The results indicate that, in general, heterodimers have greater thermal stability than homodimers and this was reflected in the estimated apparent dissociation constants. These constants for homodimers and for heterodimers were within the low µMolar range and nMolar, respectively, as previously shown. In an attempt to understand the structural basis for this preference molecular modelling studies and sequence analysis were performed. These analyses suggest the presence of a large number of charged residues in the a position of the coiled coil as being, at least partially, responsible for selectivity. Thus, these results indicate the crucial or cooperative action of C-terminal domains in the preferential organization of septins during filament assembly, specifically favouring the NC interface between septins of groups II and IV.

Key words: Septins. Coiled coil. Homo- and hetero-interactions.