EXPRESSION, PURIFICATION AND STRUCTURAL STUDIES OF HUMAN SEPTINS 6 AND 8

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Mammalian septins comprise a family of 13 genes that encode GTP-binding proteins. They are involved in cellular processes such as vesicle trafficking and exocytosis. An increasing body of data implicates the septin family in the pathogenesis of diverse disease states including neoplasia, neurodegenerative conditions and infections. All septins share a highly conserved structure comprising a variable N-terminal and C-terminal, and a conserved domain related to those found in small GTPases. Regions corresponding to the GTPase and C-terminal domains of Human septin 8 (Sept8gc) were cloned together in expression vector pET28a, expressed and purified in Escherichia coli BL21(DE)C43. Septin 8gc was purified by affinity chromatography followed by a gel filtration chromatography (superdex 200 HR 10/30). The GTPase domain of septin 6 was cloned in expression vector pET28a and expressed in E. coli BL21(DE)C43 but suffers from severe aggregation, which is prevented by purifying this septin in a specific buffer containing glycerol and GTP. This protein was purified by affinity chromatography (Ni-NTA resin) followed by a gel filtration chromatography (superdex 200 HR 10/30). The secondary structures were analyzed by Circular dichroism spectroscopy and both proteins were submitted to initial crystallization screenings using the following kits: Classic, MPD, Ion, Cation, AmSO4 and PEGs from Nextal, and Wizard I and II from Emerald.

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