STRUCTURAL AND BIOCHEMICAL STUDIES OF HUMAN SEPTIN 7

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Septins are present in fungi and animals and they belong to the protein family whose GTP-binding domain is conserved. They form homo and heteroligomeric complexes which can polymerize into filaments. In mammals, septins have been associated with a variety of cell functions, such as cytokinesis and exocytosis and, also, in the development of some neurodegenerative diseases and some types of cancer. This study is part of one of the main projects of CEPID-CBME and it aims to clone, to express in Escherichia coli in soluble form and to obtain structural and biochemical information on the human protein Septin 7 (SEPT 7) and its GTPase domain. Structural studies will be done using spectroscopic techniques such as circular dichroism, fluorescence and small-angle X-ray scattering (Saxs). The Septin 7 GTPase domain gene was amplified using a human fetal brain cDNA library and synthesized oligonucleotides. The amplified fragments were cloned in the pGEM-T vector and subcloned in the pET-TEV expression vectors. Previous data obtained by us show that the protein Septin 7 GTPase was expressed in E. coli and purified in an Ni-NTA affinity column and in a size-exclusion one, in a soluble form. The analysis of spectrum of Septin 7 GTPase domain obtained showed that is protein is formed by a-helices 32%, ß-sheet 17%, ß-turns 20% and irregularly structure 31%. We have obtained a stable crystal of Septin 7 GTPase domain and study crystallography has been development by us to increase the resolution this crystal. The results of this study will add fundamental information to the structural and biochemical knowledge on human septins.

Keywords: spectroscopic techniques, filaments and structure.
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