HIGH RESOLUTION OF GTPase DOMAIN OF SEPT3 IN COMPLEX WITH GDP AND GTP

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Septins are GTPases that play an important role in the maintenance of cell structure and serve as scaffolds for recruitment of partner proteins. Septins form hetero-filaments involving two types of interface, G and NC, as observed in the crystal structure of the hetero-trimer of SEPT2-SEPT6-SEPT7, including members of three of the four different septin subgroups. The remaining subgroup is represented by SEPT3 whose structure at low resolution (2.88 Å) was described by Macedo et al (2013). Here we present high-resolution structures of the GTPase domain of SEPT3 complexed with GDP (1.83 Å) and GTP (1.86 Å). The data was collected using The X-ray diffraction facilities at the Diamond synchrotron and solved by molecular replacement. The higher resolution obtained here allows us to better define several parts of the structure including the switch I region and its associated water molecules, which contribute to the nucleotide-binding site. Furthermore, a series of salt bridges that contribute to the stability of the NC interface can be defined with greater confidence. These include previously undescribed interactions involving residues that are unique to the SEPT3 subgroup, such as Lys126, Asp209, Phe203, Ile281, Arg127, which may be partially responsible for the marked differences observed at the NC interface in SEPT3. On comparing the GDP and GTP complexes no large structural alterations are observed. This is in contrast to our previous observations with SmSEPT10 in which the β3 strand undergoes a two-residue slippage. One significant difference between the two cases is the absence of Mg2+ in the GDP complex of SmSEPT10 suggesting that metal bound to the nucleotide phosphates may be critical for controlling the structural change. Our data therefore question the importance of strand slippage for septin function, which clearly requires further investigation.

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