Poster Presentation

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Eukaryotic zinc-dependent multifunctional nuclease I

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Members of eukaryotic nuclease I family are usually zinc, magnesium or calcium dependent, relatively small (about 300 amino acids) glycoproteins with important roles in various apoptotic processes, stress response, DNA repair machinery or sustenance scavenging. They produce 5'-mononucleotides, inorganic phosphate and mononucleosides as end products, have acidic pH optima and are able to cleave different homopolymers with usually no preference for DNA or RNA. P1/S1-like nucleases, a subgroup of nuclease I family, are zinc-dependent, with phospholipase C-like fold. They can be divided to single-strand specific (eg. S1 nuclease from Aspergillus oryzae) or unspecific. TBN1 from Solanum lycopersicum (tomato) is an unspecific P1/S1-like nuclease composed of 277 amino acids with a molecular mass of 37 kDa (when fully glycosylated). TBN1 plays an important role in specific apoptotic functions and cell senescence in plants and also exhibits anticancerogenic properties [1]. For our studies TBN1 was produced recombinantly in Nicotiana benthamiana leafs. Crystals were obtained using a combination of salt and polymer. Datasets for structural analysis were collected at BESSY II (Helmholtz-Zentrum Berlin) [2]. The final model was built and refined using data to 2.15 Å resolution. TBN1 is mainly α-helical with fold stabilized by four disulfide bridges and by the catalytic zinc cluster coordinated at the bottom of the active site cleft. Three oligosaccharides bonded on the surface significantly contribute to solubility of the enzyme. Oligomerization of TBN1 is mediated by binding of a peptide chain to the active site of a neighboring molecule and can be induced by inorganic phosphate. Based on the distribution of surface residues the possible binding sites for nucleic acids with secondary structure were identified. The newly discovered phospholipase activity significantly broadens the substrate promiscuity of TBN1 [3]. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant No. EE2.3.30.0029), by the project „BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund, Grant Agency of the Czech Technical University in Prague, grant No. SGS13/219/OHK4/3T/14 and by Institute of Plant Molecular Biology, Biology Centre, AS CR, RVO:60077344.

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