[MS5-P08] Structural features and regulatory signs of plant nuclease TBN1

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Multifunctional plant nuclease I from Solanum lycopersicum (TBN1; UniProt accession no. Q0KFV0) is a 31.6 kDa, mainly a-helical glycoprotein, which plays an important role in specific apoptotic functions, vascular system development, stress response, and tissue differentiation in plants [1]. Furthermore, anticancerogenic properties of TBN1 have been revealed recently [2]. The enzyme possesses endonuclease and exonuclease-like activity on ds and ss RNA and DNA and on structured RNA, with production of monoand oligonucleotides from the 5'-end of nucleic acids [3]. Structures of wild type TBN1 and mutant N211D were solved by our group by the MR-MAD approach [4]. Molecules of TBN1 form super-helices generated by crystal symmetry in all known structures. The intermolecular contacts are provided by the active site of one molecule and a surface loop of a neighbouring molecule. This preference of main contacts in crystals suggests the way of assembly of molecules into oligomers in solution. The interaction of the active site and the surface loop is best resolved in the currently reported structure (PDB id 4JDG), where the active centre at the zinc cluster is occupied by phosphate ion. It correlates with the behavior of TBN1 in phosphate buffer, observed with dynamic light scattering. The phosphate ion binds in the same fashion as the corresponding part of a substrate analogue in the structure of Phospholipase C [5] with a highly homologous active centre. Properties of mutants, designed to modify dimerization and activity of TBN1, suggest that deliberate disruption of the loop-active site contacts by mutations limits expression of the active enzyme. Therefore, formation of TBN1 oligomers together with phosphate binding are hypothesized to have regulatory roles in apoptotic-like and senescence processes in plants. The work on this project was supported by the Czech Science Foundation, projects no. P302/11/0855 and 202/06/0757, by the Institution research plan AV0Z50510513 of the Institute of Plant Molecular Biology, Biology Centre and the Grant Agency of the University of South Bohemia in České Budějovice grant no. 143/2013/P. We acknowledge support of the Ministry of Education, Youth and Sports of the Czech Republic (grant No. EE2.3.30.0029). The authors wish to thank Dr. U. Müller of the Helmholtz-Zentrum Berlin, for support at the beam line.

References

[1] Matousek, J., Kozlova P., Orctova, L., Schmitz, A., Pesina, K., Bannach, O., Diermann, N., Steger, G., Riesner, D.,
Biol. Chem., 388, (2007), 1-13.
[2] Matousek, J., Podzimek, T., Pouckova, P., Stehlik, J., Skvor, J., Lipovova, P., Matousek, J., Neoplasma, 57, (2010),
339-348.
[3] Podzimek, T., Matousek, J., Lipovova, P., Pouckova, P., Spiwok, V., Šantrucek, J. (2011). Plant Sci. 180, 343–351.
[4] Kovaľ, T., Lipovova, P., Podzimek, T., Matousek J., Duskova, J., Skalova, T., Stepankova, A., Hasek, J., Dohnalek, J.,
Acta Cryst., D69, (2013), 213-226.

[5] Antikainen, N. M., Monzingo, A. F., Franklin, C. L., Robertus, J. D., Martin, S. F., Arch of Biochem. and Biophys.,
417, 81-86, 2003.

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