Poster Presentation

MS019.P06

Substrate recognition by non-specific zinc-dependent 3'-nucleases

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S1-P1 3'-nucleases/nucleotidases (EC 3.1.30.1) are small, mostly alpha-helical enzymes relying on the active centre formed by three zinc ions and surrounded by varied nucleotide binding sites [1]. Plants utilise such enzymes in apoptotic processes, tissue development and senescence, protozoan parasites for securing nutrients and some gram-negative bacteria employ them presumably for a similar purpose.

Only very limited data about the details of interactions of this enzyme class with nucleic acids have been known up to date. Substrate specificity is dictated by the purpose of the enzyme in a given organism. Tomato TBN1 cleaves single strand DNA, double strand DNA, RNA and structured RNA and this is reflected by the appearance of the enzyme surface [2]. The size of the cleft, the presence of nucleotide binding sites and the distribution of electrostatic potential define the enzyme specificity. In a series of crystal structures of complexes of fungal S1 nuclease (*Aspergillus oryzae*) we can see a range of nucleic acid-protein interactions [3]. S1 specificity/substrate promiscuity is connected with a particular arrangement of surface sites. A new surface substrate-binding site is found. The first complete model of single-strand DNA substrate bound to the active site cleft shows features typical for this enzyme type (see the attached Figure). S1 nuclease can be inhibited in several different ligand-binding modes. The nucleoside-binding site can undergo remodelling upon substrate/ligand binding. The critical roles of several active site residues are confirmed by mutagenesis.

A homologous S1-P1 nuclease present in *Legionella pneumophila*, a human opportunistic pathogen, has been cloned, expressed and purified. It is a single-strand-specific DNAse/RNAse with its substrate-binding pattern differing from the previously described members of the family.

The project is supported by projects CZ.02.1.01/0.0/0.0/15_003/0000447 ELIBIO, CZ.1.05/1.1.00/02.0109 BIOCEV, and CZ.02.1.01/0.0/0.0/16_013/0001776 CIISB4HEALTH provided by ERDF and MEYS, and CSF (15-05228S).

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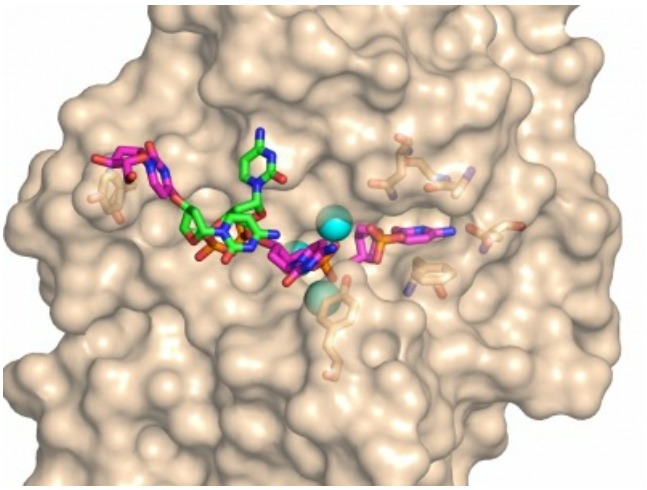

Keywords: S1-P1 nuclease, nucleic acid, substrate specificity