Structural evidence for active site complementation and diverse oligomerization in two bacterial α-L-Fucosidases from the same organism

Jan Dohnálek[1], Terézia Kovaľová[1,2], Tomáš Kovaľ[1], Jan Stránský[1], Petr Kolenko[1], Jarmila Dušková[1], Patricie Vodičková[2], Vojtěch Spiwok[2], Eva Benešová[2], Petra Lipovová[2]

[1]Institute of Biotechnology of the Czech Academy of Sciences, 25250 Vestec, Czech Republic;
[2]University of Chemistry and Technology, 166 28 Prague, Czech Republic

dohnalek@ibt.cas.cz

α-L-Fucosidases (EC 3.2.1.51) catalyse hydrolysis of the α-L-fucosyl moiety from the non-reducing terminus of oligosaccharides and glycoconjugates. New representatives are sought for their unique functional properties or particular specificity, especially in connection with the transglycosylation ability to enable targeted modification of compounds for biomedical applications. They belong to several glycosyl hydrolase families, GH29, GH95, GH139, GH141, and GH151, and utilize either the retaining or inverting mechanism. While members of some families, e.g. GH29, have been studied thoroughly, the structural information and mechanistic details for other families, including GH151, are missing.

In our previous studies [1,2] and our more recent results we bring structural and functional insights into the mechanism, active site complementation and specificity of two isoenzymes from bacterium *Paenibacillus thiaminolyticus*. The proteins were characterised using a range of biophysical techniques, small angle X-ray scattering, X-ray crystallography, and *in silico* analysis (substrate docking), together with assays of α-L-fucose hydrolysis and transglycosylation ability. The crystal structure of α-L-fucosidase isoenzyme 1 (GH29) showed a new and unusual organization of the enzyme in a hexamer, with the active sites exposed to the surrounding environment and suggested active site complementation. Mutagenesis and catalytic assays confirmed the first case of active site complementation in α-L-fucosidases [2]. Our recent crystal structure of isoenzyme 2 from the same bacterium brings the first structural insight into the GH151 family, with unexpected oligomerization, enclosure of the active site inside the oligomer, and, again, proven active site complementation. Mutations modifying the complemented amino acid lead to changes in the catalytic properties of both enzymes. The comparison on the level of structure, functional, and biophysical data for the two isoenzymes brings answers to some principal questions regarding α-L-fucosidase substrate specificity and raises new questions about the functionality and stability of complemented active sites within these families of α-L-fucosidases.

1. Benešová E, Lipovová P, Krejzová J, Kovaľová T, Buchtová P, Spiwok V & Králová B (2015) α-L-Fucosidase isoenzyme iso2 from *Paenibacillus thiaminolyticus*. *BMC Biotechnol* 15, 36.

2. Kovaľová T, Kovaľ T, Benešová E, Vodičková P, Spiwok V, Lipovová P, Dohnálek J (2019) Active site complementation and hexameric arrangement in the GH family 29; a structure–function study of α-L-fucosidase isoenzyme 1 from *Paenibacillus thiaminolyticus. Glycobiology,* 29(1), 59–73.

Keywords: α-L-Fucosidase, GH151, glycosyl hydrolase, crystal structure, active site complementation

This work was supported by the Czech Academy of Sciences (86652036), by the European Regional Development Fund (CZ.02.1.01/0.0/0.0/15_003/0000447, CZ.02.1.01/0.0/0.0/16_013/0001776, CZ.1.05/1.1.00/02.0109), by the Ministry of Education, Youth and Sports of the Czech Republic (LM2015043 and LM2018127, support of Biocev-CMS – Crystallization, Biophysics and Diffraction facilities of CIISB, part of Instruct-ERIC).