DOI 10.18699/MM-HPC-BBB-2018-08

Fighting celiac disease: improvement of pH-stability of Cathepsin L by computational design

A. Chugunov[1, 2]*, D. Nolde[2], V.F. Tereshchenkova[3], E.A. Dvoryakova[4], I.Yu. Filippova[3], E.N. Elpidina[4], R. Efremov[1, 2]

[1] *National Research University Higher School of Economics, Moscow, Russia*

[2] *M.M. Shemyakin & Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia*

[3] *Chemical Faculty and* [4] *A.N. Belozersky Institute of Physico-Chemical Biology of M.V. Lomonosov Moscow State University, Moscow, Russia*

** e-mail: batch2k@yandex.ru*

Key words: cathepsin L, pH-stability, molecular dynamics, protein design, *in silico* mutations

Motivation and Aim: Celiac disease is genetically predisposed autoimmune disorder that is caused by inflammatory response to prolamins – storage proteins of cereal seeds. Several prolamins peptides, resistant to proteolysis by human digestive enzymes, cause chronic diarrhea, abdominal distention, and even cancer and early death in susceptible human population. The common treatment is a strict wheat-, rye- and barley-free diet, known as gluten-free, which is costly and difficult to maintain.

We suggest to help celiac patients by oral treatment with enzyme that is able to effectively hydrolyze the toxic prolamins peptides – cysteine peptidase cathepsin L from a beetle *Tribolium castaneum* (TcCathL). However, this enzyme is active at pH > 3, while the use in human stomach requires it to be active at pH's as low as 2. In this work, we aimed to improve TcCathL pH-stability by *in silico* mutagenesis and computational assessment of candidate mutant variants.

Methods and Algorithms: We built a 3D homology model of TcCathL and its point mutants, and assessed their stability and dynamic features by molecular dynamics (MD) simulations in water at pH values 2 and 7, modeled as different ionization states of particular amino acid residues. Total MD time for all systems exceeded 5 μs. Processing of MD data included RMSD/RMSF calculations, analysis of intermolecular contacts, secondary structure elements stability, rotameric states of catalytic residues, etc.

Results: The major feature that distinguished TcCathL in acidic/neutral medium was structure and dynamics of the "catalytic triad": Cys-138, His-275 and Asn-295, namely – the rotameric state of His-275, which reproducibly "turned away" from the active site in multiple MD trajectories at pH 2. This peculiarity may be the cause of the loss of the activity at acidic conditions.

Next, we introduced several *in silico* point mutations in the vicinity of His-275 in order to fix its side chain in the "active" conformation by introduction of the novel hydrogen bond, and assessed these enzyme variants by MD. Several "designed" mutants of adjacent to His-275 residues exhibited the intended behavior, and were passed to the experimental verification.

Conclusion: By the computational design we suggested TcCathL mutant variants that may possess increased activity at pH 2. If so, these bioengineered enzymes become a basis for prototypic celiac disease treatment.

Acknowledgements: This work was supported by the Molecular and Cell Biology Program of the Russian Academy of Sciences, by RFBR-National Intellectual Development grant No. 17-34-80158 mol_ev_a and within the framework of a subsidy by the Russian Academic Excellence Project "5-100". Access to computational facilities of the Supercomputer Center "Polytechnical" at the St. Petersburg Polytechnic University is greatly appreciated.