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Molecular dynamics of a highly secreted α-L-arabinofuranosidase (GH62) from Aspergillus nidulans grown on sugarcane bagasse

Authors: Fabricio Bracht, Fabiano J. Contesini, Fabio M. Squina, Andre R. L. Damasio, Munir S. Skaf

Address: Universidade Estadual de Campinas, Instituto de Química, Campinas - SP

Arabinases or arabinofuranosidases are the enzymes capable of liberating L-arabinose from polysaccharides, arabino-oligosaccharides or from synthetic substrates, with endo or exo modes of action. An important group of these enzymes corresponds to the α-L-arabinofuranosidases (E.C. 3.2.1.55) that cleave α-(1→ 2), α-(1→ 3), or α-(1→ 5) linked L-arabinofuranosyl residues from non-reducing ends in oligo- and polysaccharides that contain arabinose [1]. In this work, we studied a recently identified a GH62 α-L-arabinofuranosidase (AnAbf62A) that was highly secreted when Aspergillus nidulans was cultivated on sugarcane bagasse. The crystal structure of the enzyme was solved showing the five-bladed β-propeller fold, which is conserved in family GH62 [2,3]. Site-direct mutagenesis produced Y312S and Y312F mutants which showed that Y312 is an important amino acid for binding the substrate as well as for enzymatic activity, and that both mutations are deleterious to catalytic efficiency of the enzyme.

In this computational study, we used molecular dynamics (MD) simulations and the well-tempered adaptive biasing molecular dynamics to address the question of how Y312 affects enzymatic substrate affinity and enzymatic kinetic parameters of the α-L-arabinofuranosidase (AnAbf62, GH62) from A. nidulans A773. A comparison of the structure of the AnAbf62wt with other crystal structures of the alpha-L-arabinofuranosidase from Streptomyces thermoviolaceus (PDB codes: 4O8O and 4O8P), showed that the loop containing Y312 has two different conformations [4]. The free energy simulations indicated that the loop containing Y312 can access different conformations separated by moderately low energy barriers. One of these conformations, comprising on a local minimum, is responsible for placing Y312 in the vicinity of the arabinose glycosidic bond, and thus, may be important for catalytic efficiency. This is the first report on the structural investigation of a GH62 from the genus Aspergillus.

Key-words: Molecular Dynamics, adaptive biasing molecular dynamics, loop conformation, free energy profile.

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