Poster Presentation

MS009.P54

Architecture of β-D-galactosidase active site as basis for enzyme engineering

Maria Zofia Rutkiewicz-Krotewicz[1], Anna Bujacz[1], Marta Wanarska[2], Hubert Cieslinski[2]
[1]*Institute Of Technical Biochemistry, Lodz University Of Technology, Lodz, Poland,* [2]*Microbiology Department, Gdansk University of Technology, Gdansk, Poland*
E-mail: maria.rutkiewicz-krotewicz@dokt.p.lodz.pl

β-D-galactosidases (βDG) are enzymes widely used in food industry to hydrolyze, naturally occurring in milk, lactose. Removal of this disaccharide benefits organoleptic characteristics of milk and dairy products by improving its sweetness and preventing "sand effect", which is especially important for texture quality of condensed milk and ice-creams. The milk with depleted level of lactose, or lactose-free one, is being used in production of some cheese species. Additionally, lactose-free products are classified as special nutrition products for people suffering from lactose intolerance. [1]

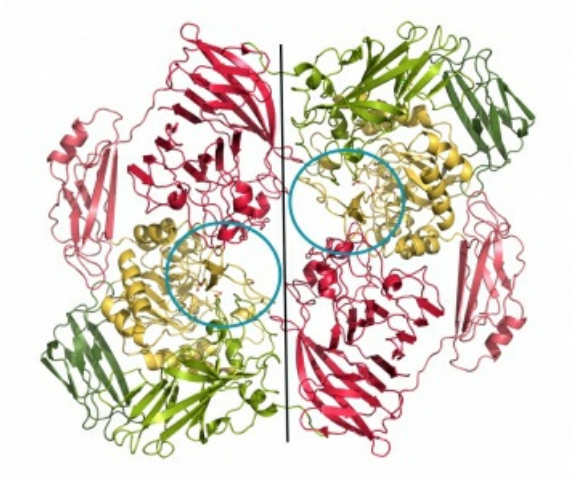
For the removal of lactose in food industry, a mesophilic βDG from Kluyveromyces lactis, which optimal temperature is 50°C, is being used. [2] However, replacing it with a cold-adapted enzyme e.g. βDG from Arthrobacter sp. 32cB [3], which is able to hydrolyze lactose at 10 °C with KM 16.56 mM and kcat 31.84 s-1, would lead to a number of advantages such as: hydrolysis of lactose during transport or storage of product, lowering the risk of mesophilic contamination (no heating of milk would be necessary), eliminating the risk of formation of unwanted products of thermal conversions, and lowering the production costs by cutting heating costs.

ArthβDG is especially interesting due to its natural transglycosylation activity - it may be used for converting of unwanted lactose into desired GOS and HOS such as lactulose - used especially in infant nourishment.

The diffraction data of complexes of ArthβDG with ligands, from crystals obtained by cocrystallization, were collected on BL14.2 line of BESSY Berlin, Germany. The data of ArthβDG complexes with: galactose, IPTG, ONPG and sucrose were processed in trigonal space group P31 2 1 up to the resolution of 2.1Å, 2.2Å, 2.8 Å and 1.9Å, respectively. Crystal structures were solved by molecular replacement using the native structure of ArthβDG as a model. Obtained crystal structures of ArthβDG complexes enabled characterization of the active site and determining residues that take part in substrate biding.

Determination of elements responsible for cold-adaptation and catalytically important residues will enable engineering of cold-adapted enzyme with enhanced transglycosylation activity.

This research was supported by grant 2016/21/B/ST5?00555 from the National Science Centre.
[1] Rutkiewicz-Krotewicz M., Pietrzyk-Brzezinska A., Sekula B., Ciesliński H, Wierzbicka-Wos A., Kur J., Bujacz A. (2016) Acta Cryst. D 72, 1049-1061
[2] Kim C. S., Ji E. S., Oh D. K. (2003) Biotechnol Lett. 25, 1769-1774
[3] Pawlak-Szukalska A., Wanarska M., Popinigis A. T., Kur J. (2014) Process Biochemistry 49, 2122-2133



Keywords: galactosidase, cold-adapted, transglycosylation