PE 4 - Natural Substrate Assay for Chitinases Using High Performance Liquid Chromatography: Determination of Kinetic Parameters

I-M. Krokeide, F.H. Cederkvist, S.J. Horn, ***V.G.H. Eijsink***, M. Sørlie

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO 5003, N-1432 Ås, Norway

Chitinases hydrolyze chitin, a β-1,4-linked polymer of N-acetylglucosamine (GlcNAc). Chitin is the second most abundant biopolymer after cellulose and, thus, represents an important natural resource. Enzymatically controlled conversion of chitin or chitosan to bioactive oligomeric compounds or to building blocks for the synthesis of bioactive glycoconjugates is of great interest. This requires the engineering of efficient chitinases with tailored substrate and product profiles. In this process, it is important to have an enzyme assay where a natural substrate is used to characterize the engineered enzymes. Until now, kinetic parameters have been determined using artifical substrates that release a fluorescent product when hydrolysed. The use of these substrates creates several problems, such as the presence of a non-natural leaving group and the occurrence of substrate inhibition, cooperative binding, and multiple productive binding modes. The challenge of kinetic analysis with a natural substrate, up till now, has been to detect sufficiently low concentrations of product and substrate, which is necessary due to the fact that Km are in the low μM range. We describe an enzyme assay using (GlcNAc)4 as substrate, combined with detection of substrate concentration disappearance using HPLC. A 2.0 mm TSD gel amide-80 column is used as a stationary phase, and 70% acetonitrile/water is the mobile phase. The substrate is hydrolysed into two dimers and hence only processed once. Assuming steady state, kcat and Km have been determined to be 28 s-1 and 4.2 μM, and 33 s-1 and 9.2 μM at t = 37 °C for chitinases A and B of Serratia marcescens, respectively, by fitting reaction rates vs. substrate concentrations in a Michaelis-Menten plot using non-linear regression.