J Proteomics Bioinform 2016, 9:12(Suppl)
http://dx.doi.org/10.4172/0974-276X.C1.095

Cloning with kinetic and thermodynamic insight of a novel hyperthermostable β-glucosidase from *Thermotoga naphthophila* RKU-10^T with excellent glucose tolerance

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With a paradigm shift in industry, moving from natural fuels to alternative renewable resource utilization, the need of efficient thermostable cellulases are expected to increase in future. β-glucosidase, an essential member of cellulases that plays a critical role in cellulosic biomass degradation and in many biological processes. Therefore, a novel β-glucosidase gene encodes a protein (*BglA*) of 446 amino acid, belonging to glycoside hydrolase family 1 (GH1), was cloned from a hyperthermophilic bacterium *Thermotoga naphthophila* RKU-10^T and over-expressed in *Escherichia coli* BL21 CodonPlus. An extracellular *BglA* with a molecular weight of 51.50 kDa, was purified to homogeneityby ion-exchange and hydrophobic interaction chromatography after heat treatment. Purified enzyme displayed optimal activity at pH 7.0 and 95°C. It was quite stable over a broad range of pH (6.0-9.0) and temperature (60-90°C), fairly stable up to 8 h at 80°C. Enzyme activity was stimulated by glucose concentration up to 600 mM and exhibited high glucose tolerance with a K_i value of 1200 mM.BglA showed great affinity towardsp-nitrophenyl substrates and cellobiose. The K_m, V_{max} and K_{cat} values, against pNPG as substrate, were 1.5 mM, 297 mmol mg^{-1}min^{-1} and 1527778 s^{-1}, respectively.Thermodynamic parameters for pNPG hydrolysis by BglA like ΔH^*, ΔG^* and ΔS^* were calculated at 95°C as 25.7 kJ mol^{-1}, 47.24 kJ mol-1 and -58.6 J mol-1 K-1, respectively. It displayed a half-life (t1/2) of 5.21 min at 97°C with denaturation parameters of enzyme including ΔH^*D, ΔG^*D and ΔS^*D were 662.04 kJ mol^{-1}, 110.10 kJ mol^{-1} and 1.491 kJ mol^{-1} K^{-1}, respectively. This is the first ever report on a highly glucose and thermotolerant β-glucosidase from Thermotoga naphthophila with high catalytic efficiency and low product inhibition, also exhibited independence of detergents and metal cations. All these significant features make *BglA* an appropriate candidate for biotechnological and industrial applications.

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Refolding of bacterially produced recombinant protein

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Recombinant therapeutic proteins when over-expressed in a suitable host like E. coli they tend to form aggregate of protein called as Inclusion Bodies (IB). IBs are inactive in nature but highly purified form of protein. IB formation can be in cytoplasmic or Periplasmic space. Mostly IB formation occurs in Cytosol. These IB are then isolated from host either by mechanical or chemical method. Isolated IB is purified using detergent. Purified IB is denatured using various denaturants and then refolded using various methods. Various traditional and Conventional method are attempted to refold the protein back to its active confirmation. Methods like dilution, dialysis, three phase partitioning (TPP), smart polymer affinity precipitation and nanoparticles are highly recommended method for refolding. In dilution and Dialysis, the protein is refolded by the gradual decreases in denaturing concentration and using refolding additives. In TPP, the protein is partitioned between an aqueous layer and organic layer using t-butanol as organic solvent and ammonium sulphate precipitation. Smart polymer precipitation uses the principle of stimuli sensitive polymer precipitation in response to stimulus. The polymer sensitive to a stimulus (pH; chemical) is incubated with protein (IB), the polymer is precipitated applying stimulus. Due to precipitation, the polymer forms complex with protein and the protein is refolded. Refolded protein is obtained by again applying the stimulus. In Nanoparticles, the principle is same as Smart Polymer, instead nanoparticles are used and there is no response to stimulus. The refolded protein is analysed for its specific activity against its specific substrate and compare with the natively expressed protein in order to know the extent of refolding.

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