An analysis of the effect of the galactomannan binding ability of β-mannosidase, BtMan2A, on its activity and synergism with a β-mannanase

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Both β-mannanases and β-mannosidases are required to degrade the mannan backbone into mannose. In this study, two β-mannosidases of glycoside hydrolase (GH) families 2 (BtMan2A) and 5 (CmMan5A) were evaluated for their substrate specificities and galactomannan binding ability, and then evaluated for their synergism with a β-mannanase, CcManA, during galactomannan degradation. CmMan5A was more active on galactose-containing oligomers and galactomannans, while BtMan2A was more active on pNPM. There existed reversible and irreversible binding of the β-mannosidases on galactomannan, with BtMan2A exhibiting irreversible binding to the greatest extent. BtMan2A binding to galactomannan did not affect its activity on pNPM, while CmMan5A binding on galactomannan significantly abolished its activity against pNPM. BtMan2A binding was pH dependant, with higher binding observed at low pH levels. Docking studies showed that BtMan2A galactomannan binding was stronger under acidic conditions (-8.4 Kcal/mol) than in a neutral environment (-7.6 kcal/mol). Furthermore, molecular dynamics simulations showed the galactomannan ligand to be more unstable in the BtMan2A binding pocket under neutral conditions than acidic conditions. Qualitative surface plasmon resonance (SPR) facilitated a comparison of the interaction between BtMan2A and galactomannan under these two pH environments, revealing reduced binding capacity at pH7. Synergistic β-mannanase to β-mannosidase (BtMan2A or CmMan5A) ratios required for maximal release of both reducing sugar and mannose from galactomannan was determined. The 75: 25% combination of CcManA with CmMan5A released more reducing sugars (1.2-fold) than CcManA alone, while combinations of CcManA with BtMan2A (≈1.0-fold) yielded no improvement. In conclusion, the low specific activity of BtMan2A towards long and galactose-containing oligomers and its non-catalytic galactomannan binding ability led to no synergy with the mannanase, making GH2 mannosidases ineffective for use in cocktails for mannan degradation.

Keywords: β-Mannanase; β-Mannosidase; Galactomannan; Non-catalytic binding; Synergy.