Understanding of the Sabatier Principle in Granular Starch Hydrolysis by Starch Binding Domain Fusions of a Psychrophilic α-amylase

Yu Wang [1], Yu Tian [3], Yuyue Zhong [3], Mohammad A. Suleiman [1], Georges Feller [5], Peter Westh [4], Andreas Blennow [3], Marie S. Møller [2, *], Birte Svensson [1, *]

1: Enzyme and Protein Chemistry, Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark

2: Applied Molecular Enzyme Chemistry, Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark

3: Department of Plant and Environmental Sciences, University of Copenhagen, DK-1871, Frederiksberg C, Denmark

4: Interfacial Enzymology, Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark

5: Laboratory of Biochemistry, Center for Protein Engineering-InBioS, University of Liège, B4000 Liège-Sart Tilman, Belgium

[*] Corresponding authors:

Marie S. Møller: msmo@dtu.dk

Birte Svensson: bis@bio.dtu.dk

Abstract

Fusion with two different starch binding domains (SBDs) of carbohydrate binding module family 20 (CBM20) conferred the psychrophilic α-amylase *Alteromonas haloplanctis* A23 (AHA) with enhanced activity towards starch granules. The SBD from either *Aspergillus niger* glucoamylase (SBD_{GA}) or *Arabidopsis thaliana* phosphoglucan, water dikinase (SBD_{GWD3}) was C-terminally merged via a 10-residue designed peptide linker to AHA. While the two AHA fusions, AHA-SBD_{GA} and AHA-SBD_{GWD3}, essentially maintained the activity towards different soluble α-glucan polysaccharides and cyclodextrins, they displayed up to 3-fold increased activity for a range of different starch granules. The interfacial catalysis was analyzed towards waxy maize starch (WMS), normal maize starch (NMS) and three maize starches with increasing amylose contents (G-50, G-80, AE) by using a combined conventional and inverse Michaelis-Menten approach. Generally, the SBD-fusion resulted in reduced $^{conv}K_m$ and increased $^{conv}k_{cat}$ values and up to 3-fold increase in $^{conv}k_{cat}/^{conv}K_m$ compared to AHA. Depending on the starch type, the $^{inv}K_m$ increased up to 3-fold, while the $^{inv}k_{cat}$ increased by 4−10 fold. Remarkably, the density of binding sites and attack sites of AHA-SBD_{GA} and AHA-SBD_{GWD3}, although decreasing 2−3 fold from the WMS through the AE starch granules, overall the density of binding sites was 3−7 fold and of attack sites 2−5 higher, than for AHA. The relationship between $^{conv}k_{cat}$, $^{inv}k_{cat}$ and $\Delta\Delta G°$ (Relative standard free energy of enzyme-substrate binding) showed that k_{cat} is negatively correlated with $\Delta\Delta G°$, which means that the degradation of the starch granules by AHA and the SBD-fusions are adsorption limited reactions according to the Sabatier principle.

Acknowledgements

This work was supported by a China Scholarship Council (CSC) grant #202006790033 (to YW) and the Technical University of Denmark.