New insights into structure/function relationships in plant \( \alpha \)-amylase family GH13 members

1Enzyme and Protein Chemistry, Søltofts Plads, Technical University of Denmark, DK-2800, Lyngby, Denmark, 2Department of Biochemistry, Faculty of Sciences, University of Debrecen, H-4010 Debrecen, Hungary, 3Laboratoire de BioCristallographie, Institut de Biologie et Chimie des Protéines, UMR 5086-CNRS/Université de Lyon, F-69367 Lyon, France

B. Svensson1, J. M. Andersen1, M. B. Vester-Christensen1, J. M. Jensen1, E.-S. Seo1, M. M. Nielsen1, J. A. Mótyán2, L. Kandra2, G. Gýemánt2, R. Haser1, N. Aghajari1, M. Abou Hachem1

Two polysaccharide binding surface sites have enormous impact on barley \( \alpha \)-amylase 1 in action on raw starch. Furthermore, SPR showed one of the sites to bind \( \beta \)-cyclodextrin 20-fold tighter than the other, which was critical in starch granule binding. Both sites influence processive hydrolysis of amylose. Noticeably preferred binding to a surface site in AMY1 of \( \beta \)-cyclodextrin differed from that of maltooligosaccharides to the catalytic site mutant Asp180Ala. Subsite maps of surface site mutants differed from wild-type.

Recent production of recombinant barley limit dextrinase inhibitor and limit dextrinase allowed SPR analysis showing sub-nanomolar complex formation driven by a slow \( k_{\text{off}} \) and hydrophobic rather than electrostatic forces.

A novel generally applicable procedure involving EDTA titration, ITC and modeling is developed to determine binding affinities of 3 structural Ca\(^{2+}\) ions to domain B of AMY1. A fitting procedure revealed Ca\(^{2+}\) binding cooperativity. The mutant K130E near one Ca\(^{2+}\) site increased Ca\(^{2+}\) affinity and thermal stability, whereas T129R had the opposite effect reflecting importance of electrostatic interactions.

Supported by the Danish Natural Science Research Council, the Danish Research Council for Technology and Production Sciences, the Carlsberg Foundation and DTU Ph.D. stipends.