

OE 7 - Processivity and Endo- Versus Exo-mechanism in Family 18 Chitinases Produced by *Serratia marcescens*

S.J. Horn⁽¹⁾, A. Sørbotten⁽²⁾, B. Synstad⁽¹⁾, P. Sikorski^(2,3), M. Sørli⁽¹⁾, K.M. Vårum⁽²⁾, **V.G.H. Eijsink⁽¹⁾**

⁽¹⁾Department of Chemistry, Biotechnology and Food Science, the Norwegian University of Life Sciences, P.O. Box 5040, 1432 Ås, Norway - ⁽²⁾NOBIPOL, Department of Biotechnology, the Norwegian University of Science and Technology, NTNU, 7491 Trondheim, Norway - ⁽³⁾Biophysics and Medical Technology, Department of Physics, NTNU, 7491, Trondheim, Norway

We have compared the enzymatic properties of ChiA, ChiB, and ChiC, the three family 18 chitinases produced by *Serratia marcescens*, using a variety of substrates, including oligosaccharides, chitin and chitosan. All three enzymes eventually converted chitin to N-acetylglucosamine dimers (GlcNAc₂) and a minor fraction of monomers. ChiC differed from ChiA and ChiB in that it initially produced longer oligosaccharides from chitin and had lower activity towards an oligomeric substrate, GlcNAc₆. ChiA and ChiB could convert GlcNAc₆ directly to three dimers, whereas ChiC produced equal amounts of tetramers and dimers, suggesting that the former two enzymes can act processively. Further insight was obtained by studying degradation of the soluble, partly deacetylated chitin-derivative chitosan. Because there exist non-productive binding modes for this substrate, it was possible to discriminate between independent binding events and processive binding events. In reactions with ChiA and ChiB the polymer disappeared very slowly, while the initially produced oligomers almost exclusively had even-numbered chain lengths in the 2 – 12 range. This demonstrates a processive mode of action in which the substrate chain moves by two sugar units at the time, regardless of whether complexes formed along the way are productive. In contrast, reactions with ChiC showed rapid disappearance of the polymer and production of a continuum of odd- and even-numbered oligomers. These results are discussed in the context of recent literature data on directionality and synergistic effects of ChiA, ChiB and ChiC, leading to the conclusion that ChiA and ChiB are processive chitinases that degrade chitin chains in opposite directions, while ChiC is a non-processive endochitinase.