

ENZYMOLOGY OF CHITOSAN HYDROLYSIS AND ANALYSIS OF THE OLIGOSACCHARIDES

Martin G. Peter

Institute of Chemistry, University of Potsdam, P.O.Box 601553, D-14415 Potsdam,
Germany

E-mail: peter@chem.uni-potsdam.de

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Chitooligosaccharides are β -1,4 linked homo- or heterooligomers of 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and/or 2-amino-2-deoxy-D-glucopyranose (GlcN). These compounds - which show intriguing biological activities - are formed in animals, fungi, and some bacteria either by biosynthesis or by enzymatic degradation of the aminoglucans chitin or chitosan (for a review, see¹).

With focus on the enzymatic degradation of chitin and Chitosan, a review on structures and mechanisms of chitinases belonging to family 18 glycosyl hydrolases is given. Comparison of several family 18 chitinases shows remarkable differences in the molecular organization of the polysaccharide binding domains as well as the substrate binding sites.

Chitin oligosaccharides composed of GlcNAc (short notation in sequences: A) or GlcN (short notation in sequences: D) were obtained after enzymatic hydrolysis of chitosan [F_A 0.19] with a fungal chitinase and separated by gel permeation chromatography. Oligosaccharides were derivatized by reductive amination with 2-aminoacridone (AMAC) and sequenced by MALDI TOF post source decay (PSD) mass spectrometry.² The sequence of a trimer, D1A2, was established as D-A-A. The composition of a hexamer D3A3 was 65 % D-A-D-D-A-A and 35 % D-D-A-D-A-A. PSD MS of a nonamer D5A4-amac revealed four isobaric species D-X-Y-D-X-Y-D-A-A, where A is GlcNAc, D is GlcN, and X and Y are mutually either D or A. This structure motif was

also observed in a dodecamer D7A5 which was composed of eight isobaric sequences of the general formula (D-X-Y)₃-D-A-A. Another dodecamer of the composition D8A4 showed a much higher diversity of structures, with variable sequences at the reducing and invariably D located at the non-reducing end.

It was concluded that the formation of the oligosaccharides occurred by a chitinase/chitosanase-like enzymatic activity and possibly also transglycosylation. Furthermore, it was concluded that the chemical deacetylation of chitin leads to chitosan with a random distribution of A residues though some regular sequence patterns were observed.

References

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