PE 3 - Identification of a High-Affinity Binding Oligosaccharide by (+) Nanoelectrospray Quadrupole Time-of-flight Mass Spectrometry of a Non-covalent Enzyme-ligand Complex

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Oligosaccharides are of current interest as targets for the development of novel pharmaceuticals and plant growth regulators. Heterochitooligosaccharides (composed of N-acetylglucosamine (GlcNAc, or A) and glucosamine (GlcN, or D) have a wide variety of biological activities, such as promotion of chondrocyte growth in cell culture and morphogenetic activity in vertebrates, or elicitor action in plants. The entities used for biological studies are usually prepared by enzymatic hydrolysis of the aminoglucan chitosan, yielding intractable, complex mixtures of aminoglucan oligomers which differ in DP (degree of polymerisation), as well as in the mole fraction of A residues (i.e. homologs) and in the sequences of D and A residues (i.e. isomers). The molecular mechanisms of the biological actions of hetero-chitooligosaccharides are essentially unknown, as complexes with receptors or enzymes have so far not been described, despite the fact that several protein-ligand complexes with GlcNAc homooligomers as well as inhibitors were investigated by protein crystallography and 1H NMR spectroscopy. We report on a study of the specificity and stoichiometry of enzyme-ligand recognition, using (+) nanoESI MS of a non-covalent protein-ligand complex. A novel methodology based on CID MS/MS was employed for top-down sequencing of the bound ligand from the non-covalent complex which was detected by nanoESI MS in the electrospray ion source.