STRUCTURE-FUNCTION RELATIONSHIPS OF PARTIALLY ACETYLATED CHITOSANS

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Chitin is one of the most abundant natural polymers, and its partially de-*N*-acetylated counterpart chitosan is one of the most promising and most versatile renewable resources. However, in spite of superior material properties and diverse biological functionalities, chitosan-based products were slow to enter the market due to poor reproducibility in production processes and product performances. Plants treated with chitosan may become resistant to disease, and even severe wounds may heal without formation of scars under chitosan dressings, but these effects were not reliably observed. More than a decade ago, we hypothesised that this failure to achieve reproducible bioactivities was at least partly due to the rather poor characterisation and the resulting batch-to-batch differences in what we now call 'first generation' chitosans.

We therefore explored in molecular or nanoscale detail structure-function relationships of partially acetylated chitosans. We analysed the influence of the degree of polymerisation (DP) and acetylation (DA) of chitosans on their antimicrobial and plant strengthening activities. We showed that chitosans with low DA and intermediate DP exhibited the highest antimicrobial activities, independent of the bacteria or fungi studied, while chitosans with intermediate DA and high DP were best for plant strengthening, but the optimal chitosan differed between plant species and disease.

When previously, 40 kg of raw chitosan was required per hectare to achieve reliable crop protection, we reduced this to 40 g/ha when using well defined and specifically optimised chitosans. We have since further improved the performance of these 'second generation' chitosans and developed two plant strengtheners which have been successfully introduced into the market.

When we tried to extend these studies to the understanding of structure-function relationships of chitosans towards human cells, even our well defined chitosans failed to give reproducible results. We assumed that the poor solubility of chitosans at neutral pH was at least partly responsible for these problems, and began to investigate the influence of nano-formulations on biological activities of chitosans.

Another approach to counter the solubility problem is to focus on chitosan oligomers rather than polymers. This became paramount when we identified chitotriosidase as the first human chitosanolytic enzyme. We found that its activity is dependent on the presence of two adjacent acetylated glucosamine residues, prompting us to postulate that the pattern of acetylation (PA) of chitosan will also crucially determine its biological activity if a sequence specific chitosan hydrolase is present in the target tissue.

However, as all chitosans available today are produced from chitin using chemical means, i.e. either partial homogeneous or heterogeneous de-*N*-acetylation, or full de-*N*-acetylation followed by partial re-*N*-acetylation, they invariably have random PA. We are, therefore, currently developing tools for the biotechnological preparation and mass spectrometric fingerprinting analysis of bio-engineered chitosans with controlled, non-random PA using recombinant chitin and chitosan synthesising and modifying enzymes in a cell factory approach. These novel 'third generation' chitosans are now being tested for their biological activities towards diverse microbial, plant, and human cells.

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