Both enzymes and chemistry can produce acetylation patterns in chitosans

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The properties and bioactivities of chitosans are governed by their structural characteristics. Whereas most chitosans used in research and applications within the last decades have not been characterized at all regarding their structure, today, the awareness for its importance is increasing. This gave rise to currently used second generation chitosans which are well-defined in terms of their degree of polymerization and their fraction of acetylation. However, the third parameter has mostly been neglected so far: the pattern of acetylation.

For many years, we have been convinced that enzymes are the only way to obtain chitosans with non-random patterns *in vitro* or *in vivo*. These chitin deacetylases (CDAs) remove specific acetyl groups from chitin or from highly acetylated chitosans. Different CDAs possess different modes of action on their substrates, resulting in products of different patterns. Lately, we have established three intertwining effects which can be used to describe the mode of action of a CDA. These could be already applied in a study on CDA3 of the fungus *Cryptococcus neoformans*, a human pathogen of global importance. Cryptococcal CDAs are of special interest, as growing data indicates that the chitin-to-chitosan conversion in the cell wall of *C. neoformans* is important to evade human immunity, turning the fungal CDAs into pathogenicity factors. Potentially, this evasion is not only facilitated by a lower fraction of acetylation, but also by a controlled pattern of acetylation of the cell wall chitosans.

Recently, our novel analytical methods revealed a surprisingly regular pattern in certain chemically produced chitosans, thus contradicting the hypothesis that only CDAs can produce non-random patterns. Whereas chitosans produced by chemical *N*-acetylation or homogeneous deacetylation possess random patterns, those produced via heterogeneous deacetylation feature a pattern where acetylated units are overrepresented at every third position. Furthermore, we found that these chitosans are much stronger elicitors of immune responses in both plants and human cells. Our works on patterns resulting from both enzymatic and chemical deacetylation highlight the importance to consider this structural parameter when establishing structure-function relationships of chitosans.