Substrates and Products of Chitin- and Chitosan-Modifying Enzymes

Nour Eddine El Gueddari, Andreas Schaaf, Markus Kohlhoff, Christian Gorzelanny, Bruno M. Moerschbacher

Department of Plant Biochemistry and Biotechnology, University of Münster,
Hindenburgplatz 55, 48143 Münster, Germany
moersch@uni-muenster.de

Chitosans have many potential applications in diverse fields such as biotechnology, material and food sciences, neutraceuticals and pharmaceuticals, pharmacology and biomedicine. Successful application in the life sciences, however, such as in wound healing or drug delivery, is hindered by our still fragmentary understanding of basic structure/function relationships. To investigate the dependency of biological functionalities on physico-chemical properties of partially acetylated chitosans, we have established bio-activity matrices in which we depict the influence of two basic parameters, namely the degree of polymerisation (DP) and of the degree of acetylation (DA), on different biological activities of chitosans, such as antimicrobial activities or the induction of disease resistance in plants. We demonstrated that biological activities of chitosans depend on both DP and DA. In all cases, the biological activities were greatly influenced by DA. As an example, the antimicrobial activity of chitosans increased with decreasing DA, fully de-N-acetylated chitosans being most active. In contrast, chitosans with intermediate degrees of acetylation were most effective in inducing resistance reactions in plants. In most cases, the influence of DP was less pronounced: above a certain critical threshold DP, the biological activity does not change greatly with increasing DP. A minimum DP of about 20 was required for significant antimicrobial activities, while the minimum DP required for elicitation of resistance reactions was typically around 5.
We believe that an in depth molecular analysis of the dependency of biological activities of chitosans on their physico-chemical properties requires control not only of DP and DA, but also of the pattern of acetylation (PA). As molecular recognition relies on matching surface properties between receptor and ligand, the PA of a chitosan oligomer can be expected to strongly influence its affinity to a given receptor in a target cell. Also, hydrolytic enzymes such as chitinases and chitosanases differ in their substrate specificities and cleavage mechanisms, so that they require a certain PA for polymer binding and chain cleavage. It can thus be expected that the hydrolytic enzymes present in a target tissue will define the processing and degradation of the chitosan polymers so that their biological activity will depend on their PA.
Chitin- and chitosan-modifying enzymes (CCME) such as chitinases, chitosanases, and chitin de-N-acetylases, thus, may help in understanding, predicting, and engineering chitosans with known and dependable physico-chemical properties and, hence, biological functionalities. The use of CCME might yield novel chitosans with non-random, e.g. blockwise or regular PA, in contrast to the chemical methods currently used for generating partially acetylated chitosans that lead to mixtures of chitosans with random PA. We are, therefore, cloning, expressing, purifying, and characterising such CCME concerning their substrate specificities and product patterns, to use them for the production of chitosan polymers and oligomers with other than random PA. These will eventually allow us to add PA as a third dimension to our bio-activity matrices of chitosans. On the other hand, we are investigating the chitosanolytic enzymes present in target tissues to understand their influence on the biological activities of chitosans.

chitinase, chitosanase, chitin de-N-acetylase, degree of acetylation, pattern of acetylation