

Molecular cloning and characterisation of two novel mollusc T-synthases

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Glycoprotein-N-acetylgalactosamine β 1,3-galactosyltransferase (T-synthase, EC 2.4.1.122) catalyses the transfer of the monosaccharide galactose from UDP-Gal to GalNAc-Ser/Thr, synthesising the core 1 O-glycan structure (T-antigen) of mucin-type O-glycans. These structures play very important role in animal development, immune response and recognition processes.

Molluscs are the largest and most successful groups of animals on the planet and can be found in a wide range of environments, including freshwater, marine, and terrestrial habitats. They play a significant role in many ecosystems as they are important filter feeders, decomposers but also pests in agriculture and intermediate hosts of many human and cattle parasites. Their ability to produce complex glycans is a fundamental aspect of their biology. The first mollusc T-synthase is already characterised [1] and identification of novel carbohydrate active enzymes will give a better understanding of their glycosylation abilities and help in elucidating their successful adaptation and survival abilities.

The sequences of the enzymes were identified by homology search using the *B.glabrata* T-synthase sequence (QXN57605.1) as a template. The genes code for transmembrane proteins with two putative N-glycosylation sites for *Pomacea canaliculata* and one putative N-glycosylation site for *Crassostrea gigas*. The coding sequences were synthesised and expressed in Sf9 cells. The expression product of the putative enzymes displayed core 1 β 1,3-galactosyltransferase activity using pNP- α -GalNAc as the substrate. These enzymes showed similar biochemical and structural parameters with previously characterised T-synthases from other species

In this study, we present the identification, cloning, expression and characterisation of the glycoprotein-N-acetylgalactosamine β 1,3-galactosyltransferase from *Pomacea canaliculata* and *Crassostrea gigas* in comparison with the previously characterised one from *B. glabrata*.

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