USING GLUCAN WATER DIKINASE FOR IN VITRO GLUCAN PHOSPHORYLATION

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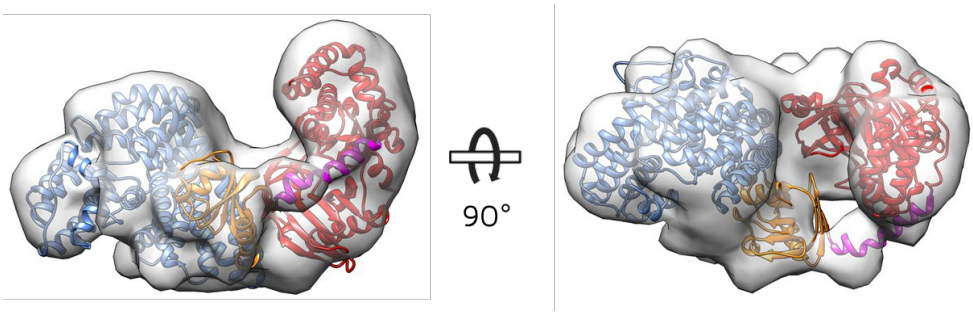
Polysaccharides are attracting growing interest in modern society because they are biosourced and biodegradable but they often lack interesting properties compared to more competitive petroleum-based polymers. The introduction of new functions on their backbones makes it possible to diversify their structures and physico-chemical properties, giving them new advantages over synthetic polymers and opening the way to new types of applications. We have recently studied different ways of functionalizing alpha-glucans. In particular, we have focused on phosphorylation as a means of modifying alpha-glucans produced by GH13 α-transglucosylases from sucrose. Until now, like most polysaccharide functionalisation processes, phosphorylation has been carried out by chemical means, which involves the use of toxic solvents and high energy costs, and often leads to degradation of the polysaccharide chain1. To overcome these drawbacks, we studied the phosphorylation of □-glucan with *Solanum tuberosum* dikinase, known to phosphorylate starch *in planta* and to be involved in its metabolism. Various □-glucans produced with GH13 amylosucrase were successfully phosphorylated, demonstrating the value of cascade reactions combining amylosucrase and kinase for the *in vitro* synthesis of phosphorylated amylose. The recombinant form of the kinase, as well as truncated versions designed from models obtained using AlphaFold2, were studied by a combination of structural biology techniques including X-ray crystallography, SAXS and CryoEM. This enabled us to solve the first 3D-structures of several StGWD1domains and to propose a new organisation of the protein into 5 domains. Our data confirm the existence of a pivotal movement in the protein for glucan phosphorylation. They provide a better understanding of the structure-function relationships of this important enzyme and offer interesting prospects for guiding enzyme engineering and making StGWD1 more efficient for the phosphorylation of amylosucrase-derived polysaccharides as well as structurally different α-glucans.



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