Acylation of soluble polysaccharides in a biphasic system catalyzed by a CE2 acetyl esterase

Acylated sugars attain altered or improved properties comparing to their native form and thus present great commercial significance due to the fact that they can be used in several applications, such as biosurfactants in biomedicine, foods, detergents and cosmetics industry. Acylated polysaccharides are of great significance and value as biocompatible and biodegradable amphiphilic compounds. For example, the acetylated polysaccharide glucomannan from *Cyrtopodium andersonii R. Br*., has been proven to have anti-inflammatory and gastro-protective properties, while modified pectin is used in food industry and cosmetics. Starch undergoes acylation in order to increase its hydrophobicity and is applied in plastics and food industries as well as in Biomedicine as drug delivery systems or as scaffolds.

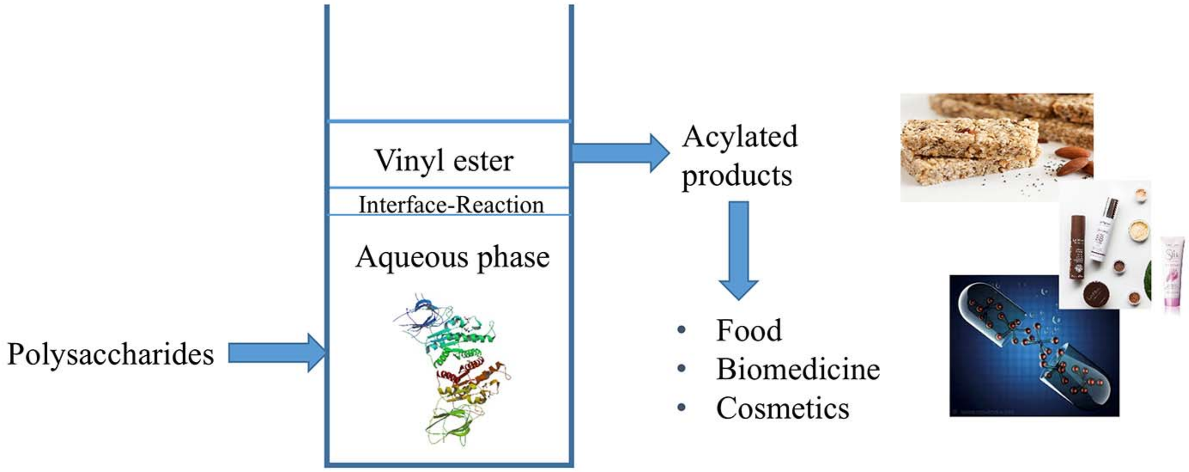

Fig. 1.

Traditionally, the acylation procedure was carried out in organic solvents that were usually catalyzed with organic or inorganic catalysts which, however, presented disadvantages due to high temperatures and low selectivity that results to the formation of side products. Taking these into account, we have turned into the search of enzymatic procedures aiming at the acylation of sugars owing to their benevolent environmental footprint and their regioselectivity and specificity as opposed to chemical synthesis. A carbohydrate esterase of family 2 from *Clostridium thermocellum, Ct*CE2, has been proven that as far as the hydrolytic and synthetic activity is concerned, it features regioselectivity towards 6-O position of glucopyranosides. Along with the use of biocatalysts, the possibility to have a reaction system of aqueous nature has been intriguing and consists an innovate approach, since literature is dedicated to the use of organic solvents or ionic liquids as reaction means.

In the present work, the acylation potential of a glucomannan acetyl esterase from bacterium *C. thermocellum* (CtCE2) was investigated on β-glucan and manno-polysaccharide substrates using vinyl acetate, propionate and butyrate as acyl donors in biphasic system. The transesterification reactions were carried out in two-phase mixtures consisted of water/vinyl esters. Relying on thermodynamics it has been proved before that the ester keeps being produced enzymatically in the system interface and is being transferred in the acyl donor phase due to its low solubility in water, until equilibrium is reached. In addition, in this biphasic system, there is no need for immobilization or lyophilization of the biocatalyst or even the addition of surfactants that are required in reaction systems composed of an organic solvent, since the enzyme is protected from denaturation in the aqueous phase.

Initially, acylation reactions were performed on aldohexose monosaccharides and disaccharides in order to evaluate the potential of the enzyme using different acyl donors in the respective reaction conditions. The qualitative analysis was performed via TLC, as well as FTIR in the case of polysaccharides. The quantification of all acylated products was justified by HPLC. The acylated mono- and disaccharides, were isolated and identified with ESI–MS, while O-6 regioselectivity was proven with NMR. The CE2 esterase reached high conversion yields for the acylation of the primary hydroxyl group of aldohexose mono- and disaccharides revealing its synthetic potential. Modified monosaccharides acquired acylation yields from 11 up to 65%, showing preference for small chain acyl donors, while disaccharides exhibited conversion yields from 23 up to 58%, with preference to monoacylation. Acylation of polysaccharides were confirmed by TLC and FTIR, while the degree of acylation was determined via an indirect method, estimating a range of acylation from 0.022 to 1.083 $\mathrm{mmol}_{\text{acyl group}} * \mathrm{g}_{\text{polysaccharide}}{}^{-1}$ depending on the structure and composition of the target polysaccharide, with 1,4 β-D-mannan displaying the highest modification yields. For the first time, a member of CE2 acetyl esterase was found capable of acylating soluble polysaccharides for the production of modified natural polymers with potential use in industry.

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