

UNIQUE MODE OF ACETYLATION OF OLIGOSACCHARIDES BY *TRICHODERMA REESEI* ACETYL ESTERASE IN WATER-VINYL ACETATE MIXTURES

Lubomír Kremnický and Peter Biely*

*Institute of Chemistry, Slovak Academy of Sciences, 84538 Bratislava, Slovakia
chempbsa@savba.sk*

Acetyl esterase (AcE) from *T. reesei* RUT C-30 [1] catalyzes acetyl transfer to variety of carbohydrates in water in the presence of vinyl acetate as the acetyl group donor [2]. The degree of conversion and the number of formed acetates depended on the acceptor used. With some acceptors, such as methyl or 4-nitrophenyl β -D-glucopyranoside, only 3-O-acetyl derivative was formed in 50-70% yield [2]. In this work three series of oligosaccharides were examined as acetyl group acceptors in this transacetylation. Regardless of their polymerization degree, β -1,4-xylooligosaccharides and β -1,4-glucooligosaccharides (cellooligosaccharides) were transformed in good yields mainly to monoacetates and partially to diacetates, suggesting that only terminal sugar residues are acetylated. When methyl β -glycosides of oligosaccharides or reduced oligosaccharides were used as glycosyl acceptors, only monoacetates were formed. The formation of diacetates was never significant with β -1,4-mannooligosaccharides, indicating that the AcE does not recognize the anomeric configuration of the reducing end mannose residue. These observations suggested that the first and the main acetylation of oligosaccharides takes place at the non-reducing carbohydrate residue, and the second, slower acetylation, at their reducing end. The presence of the acetyl group at the non-reducing residue in acetylated xylo- and cellooligosaccharides was proven by their resistance to the corresponding glycosidases. Due to the acetyl group migration [3], the products of xylooligoaccharide acetylation were not uniform and therefore were not subjected to structural analysis. The acetyl group migration is apparently much slower in cellooligosaccharides since the position the of acetyl groups could be established by NMR. The main acetylation takes place at position 3 of the non-reducing glucopyranosyl residue, and the second, slower acetylation, occurs at position 2 of the reducing-end glucopyranose. The two-site „exo“-pattern acetylation of xylo- and cellooligosaccharides in contrast to one-site „exo“-acetylation of mannoooligosaccharides was also confirmed by ESI MS of the acetylation mixtures. It remains to be established whether the observed positional specificity of the enzyme in the transacetylation reaction corresponds to its regiospecificity of deacetylation [1]. Assays with monoacetates of 4-nitrophenyl β -D-xylopyranoside suggest that, in contrast to typical acetylxyylan esterases, showing preference for deacetylation of position 2 [4], the investigated *T. reesei* AcE operates better on sugar glycosides acetylated at position 3 or 4. Significant activity to 3-O- and 4-O-acetyl 4-nitrophenyl β -D-xylopyranoside in crude cellulolytic and hemicellulolytic systems of variety of microorganisms [4] suggests that the production of this type of AcE is not restricted to *T. reesei*.

[1] Poutanen K., Sundberg M. (1988) *Appl. Microbiol. Biotechnol.* 28:419-424

[2] Kremnický L., Mastihuba V., Cote G.L. (2004) *J. Mol. Catalysis B: Enzymatic* 30:229-239.

[3] Mastihubová M., Biely P. (2004) *Carbohydr. Res.* 339:1353-1360.

[4] Biely P., Mastihubová M., la Grange D.C., van Zyl W.H., Prior B.A. (2004) *Anal. Biochem.* 332:109-115.