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Freeing anomeric hydroxyl groups of peracetylated oligosaccharides by *Aspergillus niger* lipase

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Abstract—Various peracetylated oligosaccharides (di- to hepta-) are subjected to regioselective hydrolysis by *Aspergillus niger* lipase hydrolyzing 1-*O*-acetyl group. Molecular weight, anomeric and interglycosidic linkage configurations play a significant role in the hydrolysis reaction. Useful 1-*O*-deprotected material can be easily prepared for further chemical elaboration. © 2002 Elsevier Science Ltd. All rights reserved.

Lipases are used in organic synthesis to perform enantioselective hydrolytic reactions or to obtain ester or amide bond formation.[1] Among examples of applications of these enzymes with polyhydroxylated natural products (steroids, sugars, glycosides, etc.) both acylation or deacylation are possible. For the latter case we reported[2] examples where enzymatically synthesized erythritol or glycerol β-glucosides, when peracetylated, were used as substrates for PFL-mediated hydrolysis obtaining only one homochiral product and showing that sugar moieties were not affected by the enzyme. Conversely when using peracetylated mono- and disaccharides, lipases preferentially attack anomeric acyl groups. For this reaction *Aspergillus niger* lipase appears to be the biocatalyst of choice but other lipases also react in the same manner.[3] Treatment of β-cellobiose, β-maltose and α/β-lactose octaacetates with *A. niger* lipase provided the corresponding heptaacetate with a free anomeric group.[4] A lot of chemical techniques are reported in the literature for this reaction in that protected sugars having one free hydroxyl group at anomeric carbon are versatile building blocks for the synthesis of oligosaccharides and glycoconjugates.[5] Among the anomeric chemical deprotection techniques, the one using piperidine was not judged as efficient as the enzymatic process.[6] The deprotected material can be used, for example, to synthesize the corresponding *O*-methyl glycosides (via methyl iodide) in a convenient large-scale reaction[7] useful for the insertion of labelled aglycone into the products. This simple methodology is appropriate for the preparation of chromophoric oligosaccharides, substrates for the kinetic characterization of new glycosyl hydrolases, in which we are involved, or for biological studies.[7]

In this communication we report our results on the use of *A. niger* lipase for the hydrolysis of 1-*O*-acetyl groups in different examples of di-, tri- and higher oligosaccharides of interest. To the best of our knowledge this is the first report on the lipase-mediated regioselective hydrolysis of complex oligosaccharides. Different anomeric selectivities of the enzymes were evidenced (Fig. 1).

Maltose octaacetate was prepared from commercial maltose by acetylation using iodine instead of zinc chloride as Lewis acid catalyst securing high yield of α-anomer.[8] Other maltooligosaccharides were commercially available and acetylated by pyridine/Ac_2O overnight. Peracetylated laminaribiose and laminaritriose were obtained by acetolysis of the inexpensive microbial polysaccharide curdlan.[9] Isoprimeverose is the basic disaccharide unit of an important natural polymer known as xyloglucan. It was enzymatically synthesized using an archeal α-xylosidase by coupling xylosyl fluoride and glucose.[10] The related XXXG oligosaccharide was obtained from tamarind seed xyloglucan through a set of glycosyl hydrolase-mediated reactions.[10,11]

In a typical lipase reaction 50–200 mg of each substrate in acetone/20 mM phosphate buffer pH 7, 1:1 v/v (50 mg/ml) was reacted with *A. niger* lipase (Fluka, 0.2 U/mg of substrate). Following usual work-up[3,4] the remaining substrate and products were purified by silica

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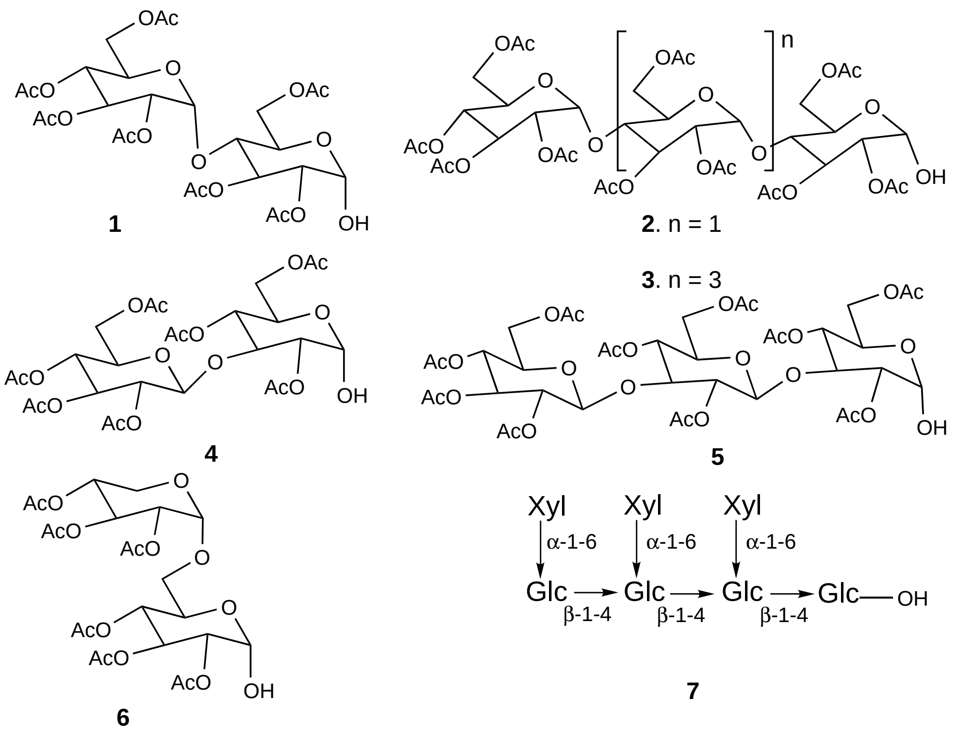

Figure 1. Oligosaccharides obtained by *Aspergillus niger* lipase hydrolysis. For compound **7** Xyl and Glc indicate the acetylated forms of these sugars.

gel flash chromatography and subjected both to mono and bi-dimensional NMR spectroscopy.[12] Anomeric ^{1}H and ^{13}C signals in the substrates are clearly distinguishable in a less crowded zone of the spectra; after hydrolysis the free anomeric ^{1}H signals shifted in a more crowded zone of the spectra are easily assigned by ^{1}H–^{13}C NMR correlation. The products are present in a predominant α-form (α/β ratio ca. 80:20).

Peracetylated malto-oligosacchararides were good substrates for the *A. niger* lipase. Substrate conversion in the reactions using these compounds reaches 100% in 30 min to 3 h for maltose and maltotriose, respectively; hence, either anomers were quickly consumed. Under the same conditions maltopentaose reaches 70% conversion after 16 h reaction. The reactions are regioselective in that the products obtained lack an anomeric acetyl group. The remaining maltopentaose shows almost the same anomeric ratio as the starting peracetylated oligosaccharide.

The β-1,3 linked disaccharide laminaribiose is also substrate for the enzyme; the conversion reaches 56% in less than 30 min; after 3.5 h no more substrate was consumed. ^{1}H and ^{13}C NMR spectra of the product show that, as in the case of maltose, the regioselectivity towards anomeric acetyl group of this disaccharide is the same. Interestingly ^{1}H NMR spectra of the remaining substrate shows that almost pure α-anomer of laminaribiose remains unaffected. Peracetylated laminaritrioses with different starting anomeric ratios were reacted under the same conditions and after 16 h 15–90% conversions were reached depending on the amount of β-anomer in the mixture. Addition of a new aliquot of the lipase is not useful to complete the reaction (entries 5 and 5a). The α/β ratio of the remaining substrate also shows in this case that only the β-anomer of peracetylated substrate is hydrolyzed.

The conversion of peracetylated isoprimeverose stops at 34% and the analysis of the α/β ratio in the remaining substrate shows an enrichment in the α-anomer. Traces of more polar compounds are detectable by TLC of this reaction leading to the conclusion that other positions in the pyranose rings could be hydrolyzed on prolonged reaction times. The peracetylated form of a more complex oligosaccharide of this type, the heptasaccharide XXXG which represents a large part of the structure of native xyloglucan,[12] was also subjected to hydrolysis by the lipase and the results clearly show that the β-anomer was selectively hydrolyzed, the remaining substrate (after 3.5 h) being the pure α-anomer.

In this communication we have focused our attention on three main factors: (i) the molecular weight of oligosaccharides subjected to the hydrolysis reaction; (ii) the influence of the nature of interglycosidic linkage(s) and (iii) the influence of the anomeric configuration at reducing ends of the molecules in the hydrolysis reaction.

The results obtained here (Table 1) clearly show that lipase from *A. niger* reacts with peracetylated oligosaccharides hydrolyzing regioselectively the 1-*O*-Ac group at the reducing end and confirming previous results.[3,4] Trisaccharides such as maltotriose and laminaritriose and high molecular weight compounds such as maltopentaose and XXXG can be also accepted as sub-

Table 1. Oligosaccharides subjected to hydrolysis by *Aspergillus niger* lipase

Entry	Peracetylated substrates	Anomeric ^{1}H/^{13}C signals of substrates (initial α/β ratio)[a]	Products	(α/β) ratio of unaffected substrates	Anomeric ^{1}H/^{13}C signals of products	Substrate conversion (%)[b]
1	Maltose	α: 6.20/88.7—β: 5.70/91.3 (85/15)	**1**	–	α: 5.30/89.9—β: 4.70/94.8	100
2	Maltotriose	α: 6.22/88.8—β: 5.73/91.2 (37/63)	**2**	–	α: 5.30/89.9—β: 4.72/94.8	100
3	Maltopentaose	α: 6.21/88.8—β: 5.72/91.4 (36/64)	**3**	27/73	α: 5.30/90.0—β: 4.72/95.6	70
4	Laminaribiose	α: 6.23/89.2—β: 5.60/91.7 (41/59)	**4**	$>$99/1	α: 5.37/89.8—β: 4.59/95.3	56
5	Laminaritriose	α: 6.21/89.1—β: 5.60/91.7 (80/20)	**5**	$>$99/1	α: 5.38/90.2—β: 4.53/95.8	15
5a	Laminaritriose	α: 6.21/89.1—β: 5.60/91.7 (35/65)	**5**	$>$99/1	–	90
6	Isoprimeverose	α: 6.30/89.9—β: 5.66/91.5 (22/78)	**6**	60/40	α: 5.42/90.0—β: 4.74/95.4	34
7	XXXG	α: 6.18/88.8—β: 5.65/91.2 (36/64)	**7**	$>$99/1	α: 5.41/90.0	$>$70

[a] As established by ^{1}H–^{13}C NMR correlations.

[b] The conversions were obtained by weight after chromatographic purification.

strates and the multitude of their acyl groups remain untouched. In the case of malto-oligosaccharides, the total conversion of the substrates depends on their molecular weights: reaction times to reach reasonable yields increase from less than 30 min for maltose to 24 h for maltopentaose. The configuration of the anomeric reducing end did not play a significant role in this case. In fact complete conversions were achieved for the maltose (enriched in the α-form) and for maltotriose (enriched in the β-form). In the case of maltopentaose an anomeric ratio almost identical to that of the substrate is detected in the remaining peracetylated compound after 24 h of reaction, confirming this view.

On the contrary, the configuration of the anomeric end of peracetylated oligosaccharides plays a significant role in the hydrolysis of β-1-3 compounds. From a comparison of the anomeric ratios of the substrates and that of the remaining peracetylated compounds after the reaction, it was shown that the β-anomer was quickly hydrolyzed. Cellobiose (β-1-4) also shows this anomeric selectivity under our reaction conditions (data not shown). The discrimination between α- and β-anomers at the reducing end of β-1-3 and β-1-4 oligosaccharides reported here for the first time could be seen as a further aspect of the role that the stereochemistry plays in these reactions; the stereochemistry of interglycosidic linkage was in fact previously related[4,6] to the phenomenon of further hydrolysis reaction at C-2 position, as observed for cellobiose and lactose (β-1-4) but not for maltose (α-1-4). The discrimination between α- and β-anomers and the results obtained here for isoprimeverose and XXXG (α-1-6) and for cellobiose indicate a more complex relationship in which global structural characteristics of oligosaccharides must be taken into account. Preliminary results on the hydrolysis of peracetylated laminaribiose using *Candida rugosa*, *Candida cylindracea* and *Candida antarctica* lipases show that this anomeric selectivity is lipase-specific and not a general characteristic. The first two enzymes preferentially hydrolyze the α-anomer, while *C. antarctica* lipase behave similarly as *A. niger* lipase. The results reported here are of great interest in the carbohydrate chemistry for the preparation of selectively deprotected material, derived from complex structures (not limited to mono- and disaccharides) for further chemical elaboration.[5] The finding that overall molecular characteristics can influence both substrate conversion and anomeric selectivity in the hydrolysis reaction and that the latter is lipase specific is very intriguing. A more systematic investigation addressing these lines, i.e. starting from mono- to oligosaccharides of different structures and using different lipases can improve the use of these enzymes in the carbohydrate field.

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11. The XXXG substrate (X = disaccharide isoprimeverose, G = glucose) was a kind gift of Dr. Mitsuishi to Dr. M. Moracci and was used by us for kinetic characterization of the archeal α-xylosidase (see Ref. 10).
12. All the signals ($CDCl_3$, 500 MHz) are in agreement with proposed structures.