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Comparison between various commercial sources of almond β-glucosidase for the production of alkyl glucosides

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Abstract

Ten different almond β-glucosidases, from eight commercial sources, were compared for their capacity to produce octyl glucoside by direct condensation from glucose and octanol, with and without added dimethyl formamide (DMF) in the octanol media. All the preparations tested catalyzed the synthesis of octyl glucoside, at rates ranging from 0.002 to 0.012 μmol/h, per added unit of enzyme, one unit being defined as the amount of enzyme catalyzing the hydrolysis of 1 μmol of *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPG) per minute at 50 °C at pH 4.8. However, none of the preparations showed strong activation by DMF, contrary to what has been previously observed. Some preparations were slightly activated (two-fold maximum) by 10% DMF. It appears that the enzymes with the lowest specific activity for the hydrolysis of *p*-NPG were activated more strongly by DMF than the most active preparations.

Keywords: Glucosidase; Almond; Glucoside; Condensation; Synthesis

1. Introduction

Medium-chain alkylglucosides, such as octyl glucoside, are mild non-ionic surfactants, difficult and expensive to produce by chemical means because of the number of protection and deprotection steps required. This has limited their use to research laboratories where they are utilized to dissolve cellular membranes without affecting proteins. Due to the high regio-, stereo- and anomeric selectivity of glycosidases, an enzymatic approach for the synthesis of such products has attracted considerable interest for several years in the context of green chemistry and sustainable development [1,2]. In the past few years, our team has studied the enzymatic production of octyl glucoside using β-glucosidases either by transglycosylation [3] or by direct condensation [4]. We reported that the presence of dimethyl formamide (DMF) greatly improved conversion rates and product yield. In particular, when performing condensation using β-glucosidase extracted from almond (*Prunus amygdalus*), the addition of 20% DMF to 1-octanol can increase octyl β-D-glucopyranoside final concentration from 40 to 100 mM with an eight-fold increase of the reaction rate at a water activity of 0.53 [4]. Moreover, almond β-glucosidase was the only tested enzyme not inhibited by glucose [3], which is an asset from a process development point of view. During the year 2001, a vast number of reactions were performed in our laboratory with the same almond β-glucosidase sold by Sigma (catalogue no. G0395, lot no. 128H4027), always leading to the same conclusions (activating effect of DMF on almond β-glucosidase activity). On the basis of these encouraging results, we tried to develop a process. Surprisingly, during the scale-up, which was performed with a different lot number, we did not observe the activating effect of DMF on the reaction rates. Faced with the problem of being unable to reproduce our previous results with a different lot of Sigma enzyme, we decided to investigate the behavior of various almond β-glucosidases from different distributors in the synthesis of octyl glucoside.

2. Materials and methods

The origin of all almond β-glucosidases used is specified in Table 1. They were provided either by Sigma-Aldrich (Oakville, Ont., Canada), Worthington Biochemical Corporation (Lake-

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Table 1
Characteristics of almond β-glucosidases from various origins (one unit of enzyme hydrolyzes 1 μmol of *p*-NPG per minute at 50 °C at pH 4.8)

Company	Cat no.	Lot no.	Name	Activity (unit/mg powder)
Sigma	G0395	128H4027	A	2.8[a]
Sigma	G0395	120K4089	B	27.2
Sigma	G0395	73K4079	C	9.5
Worthington	2198	J1K697J	D	5.2
Worthington	2198	J1K701LP	E	5.6
Seikagaku America	100-590-1	NB121855AM	F	6.0
Biozyme	GSA2	272RB	G	29.7
Toyobo	BGH-201	36250	H	53.6
Kikkoman	60126	BGA017	I	11.0
Oriental Yeast Co. Ltd.	46360903	34593303	J	37.4
USBiological	G3059	L4020503	K	5.6

[a] Measured in 2000 when performing all experiments previously published [3,4].

wood, NJ, USA), Seikagaku America distributed by BioLynx (Brockville, Ont., Canada), Biozyme Laboratories (San Diego, CA, USA), Toyobo Enzymes distributed by Shinko American Inc. (New York, NY, USA), Kikkoman Corporation (Noda-city, Japan), Oriental Yeast Co. Ltd. (Andover, MA, USA), or United States Biological distributed by TeckniScience Inc. (Terrebonne, Que., Canada).

n-Octyl-β-D-glucopyranoside and *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPG) were purchased from Sigma-Aldrich. Glucose and Amberlite XAD-4 resin were purchased from BDH (Montreal, Que., Canada). All solvents used were always from the highest grade available and were used without further purification.

Immobilization of almond β-glucosidase on Amberlite XAD-4 was performed as described previously [4].

Enzymatic synthesis of octyl glucoside in octanol was performed as previously described [4]. Before starting the reactions, the enzyme and reaction media (300 mg glucose and 2 mL of 1-octanol or a mixture of 1-octanol/DMF) were equilibrated separately in chambers containing saturated NaCl solutions to preset the water activity at 0.75.

Reactions were initiated by the addition of 70 units of almond β-glucosidase either under lyophilized form or immobilized on XAD-4 resin, and reactions were performed in closed vessels at 50 °C.

Enzymatic hydrolysis of *p*-NPG in octanol was performed as follows. Before starting the reactions, the enzyme and reaction media (*p*-NPG 35 mM in 2 mL of 1-octanol or a mixture of 1-octanol/DMF) were equilibrated separately in chambers containing saturated NaCl solutions to preset the water activity at 0.75. Reactions were initiated by the addition of 35 units of lyophilized almond β-glucosidase and reactions were performed in closed vials at 50 °C.

One activity unit was defined as the enzyme quantity required to hydrolyze 1 μmol of *p*-NPG per minute at 50 °C at pH 4.8.

HPLC analyses of *n*-octyl-β-D-glucopyranoside, *p*-nitrophenol and 1-octanol were performed on a Waters Millenium^{32} liquid chromatography system with the following conditions as described previously: reversed-phase column CSC-Inertsil 150 Å/ODS2, 5 μm, 25 cm × 0.46 cm (CSC, Montreal, Que., Canada) maintained at 25 °C; solvent system, 60:40 (v/v) acetonitrile/water; flow rate, 1 mL/min; peaks detected by a Waters 410 refractive index detector [3].

3. Results and discussion

The activity of each enzyme preparation for the hydrolysis of *p*-NPG in buffer has been measured so that the same number of units could be added in the synthesis reaction assays. The purity of the powders varies, leading to a wide range of specific activities, from 2.8 to 54 U/mg, as shown in Table 1. These various lyophilized enzyme preparations have different capacities to catalyze the condensation reaction, as can be seen in Fig. 1. The most active ones can lead to initial reaction rates of 0.012 μmol/h per *p*-NPG unit of added enzyme, while the less active one leads to 0.002 μmol/h, per added unit.

We then performed, for each lyophilized enzyme preparation, the synthesis of octyl glucoside in octanol in the presence of various concentrations of DMF, up to 20% (v/v). Not all the enzymes tested behaved alike, and they have been classified depending on three different trends we observed. For four enzymes (C, D, E, K), the addition of DMF in the media up to 10% increases the initial rate slightly (around two-fold), and more DMF in the media induces a decrease in the rate (Fig. 2a). For enzymes B, F and I, no major change in activity was observed, except a complete loss of activity in the presence of 20% DMF for enzyme B (Fig. 2b). A last category of enzyme (G, H, J) seems to be very sensitive to the presence of DMF with a continuous loss of activity as the concentration of DMF increases in the media (Fig. 2c). Unfortunately, we have not observed the previously

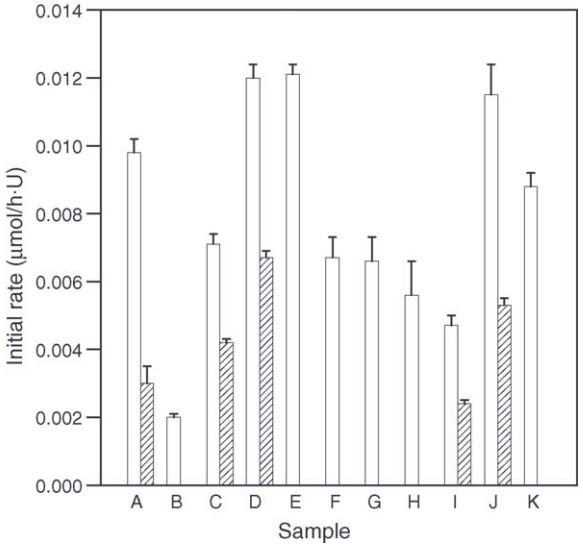

Fig. 1. Initial rates for the synthesis of octyl glucoside catalyzed by almond β-glucosidases from various origins (300 mg D-glucose, 2 mL 1-octanol, water activity preset at 0.75, 50 °C). Open bars, lyophilized enzyme; hatched bars, enzyme adsorbed on XAD-4 resin. In the case of enzyme A, the enzyme immobilized on XAD-4 and stored for 3 years at 4 °C was desorbed, re-lyophilized and tested. The initial rate for immobilized enzyme A was measured in 2000.

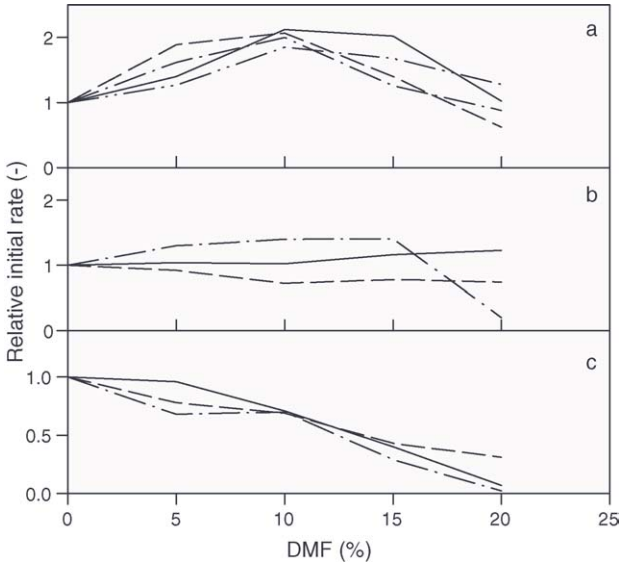

Fig. 2. Effect of DMF on the initial enzyme activity in synthesis for lyophilised almond β-glucosidases from various origins (panel a, C (–···–), D (––), E (–·–·), K (—); panel b, B (–·––·), F (—), I (––); panel c, G (—), H (–·–·), J (––)).

reported activating effect of DMF on the synthesis of octyl glucoside [4], for any of the enzymes.

To evaluate the effect of immobilization on the behavior of the enzyme in the presence of DMF, we immobilized the most tolerant enzyme preparations on XAD-4 resin. We tested them for the synthesis of octyl glucoside in the presence of various concentrations of DMF. The initial rates obtained are slightly less than those obtained for the free enzyme, as shown in Fig. 1, but trends shown in Fig. 3 are similar to those obtained with the lyophilized enzymes (Fig. 2). Again, we did not retrieve the activating effect obtained with A in 2000. As we still had some immobilized preparation of A stored at 4 °C since 2000, we decided to test it under the same conditions. More than 3 years later, the immobilized enzyme kept its activity and the

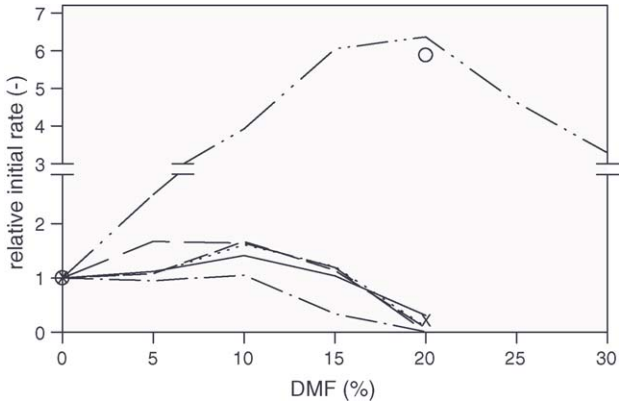

Fig. 3. Effect of DMF on the initial enzyme activity in synthesis for almond β-glucosidases from various origins immobilized on XAD-4 resin (A (–···–), B (×), C (——), D (––––), E (. . .), I (–·–·), K (—)). For enzyme A, the results were published earlier [4] and are given for comparison. The circle (◯) was obtained using this same immobilized enzyme preparation, stored at 4 °C for 3 years.

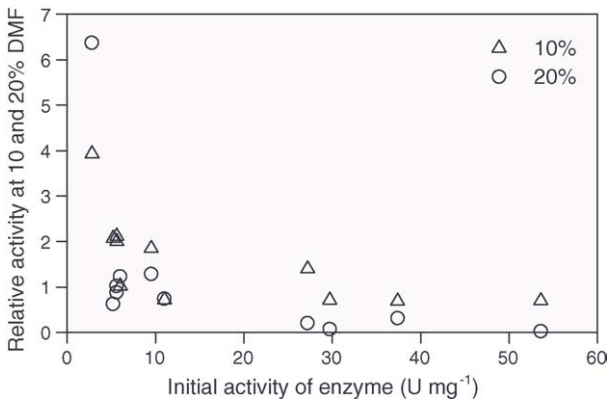

Fig. 4. Variation of the relative synthetic activity in the presence of 10 and 20% DMF, as a function of the specific activity of the commercial sample. The relative activity was calculated by dividing the rate of reaction in the presence of DMF by the rate without DMF for the same enzyme.

same DMF activation was observed, shown by the increase of the initial rate by six times in the presence of 20% of DMF (Fig. 3).

It seems there is a correlation between the purity of the enzyme powder and the activating effect of DMF for the synthesis of octyl glucoside, as seen on Fig. 4. It is known that crude extracts of almond contain many β-glucosidases [5,6]; however, these enzymes have not been characterized, apart from the fact that at least one of them belongs to family 1 of glycosidases [7]. In another member of the *Prunus* genus, *Prunus serotina* (black cherry), it has been shown that the seeds contain up to seven β-glucosidases, and that the level of expression of some of these enzymes can even vary from seed to seed [8]. This heterogeneity could explain the results on solvent activation. It could be possible that when the mixture of enzymes is purified, the enzyme(s) more tolerant to DMF is (or are) eliminated. Another possible explanation is that some small molecule (peptide or secondary metabolite), interacting with the enzyme and protecting it from the solvent, is lost upon purification. This would also explain the fact that we did not recover the solvent activation in the original lot from Sigma (enzyme A) after desorption from XAD-4 and freeze-drying (Fig. 5).

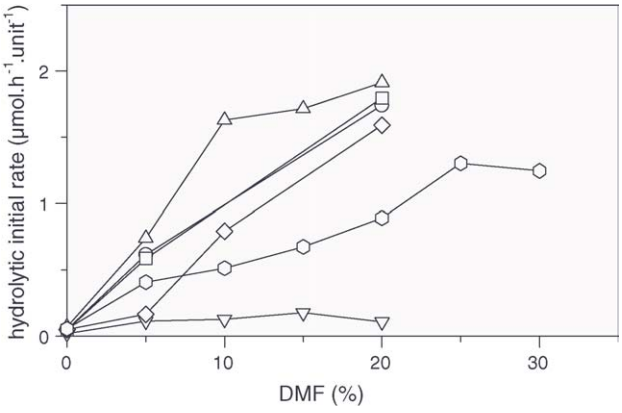

Fig. 5. Effect of DMF on the initial rate of *p*-NPG hydrolysis in 1-octanol for lyophilized almond β-glucosidases from various origins. A (⬡), B (▽), C (◊), D (◯), E (□), K (△).

We also measured the effect of DMF on the hydrolysis of *p*-NPG in octanol at $a_W = 0.75$ for some lyophilized enzyme preparations. Because of the number of different preparations and conditions, complete kinetic determinations were not performed, but only initial rate measurements at 35 mM *p*-NPG. This concentration corresponds to 4.5- to 10-fold the value of Km for *p*-NPG in water for enzyme A from Sigma, with 0–20% DMF. It also corresponds to the solubility of *p*-NPG in octanol, when no DMF is added. Contrary to what was observed when DMF was added to water [9], the rate of hydrolysis increases when DMF is added for five of the six preparations. The enzyme not showing an increase in the initial hydrolysis rate is one of the three that were little affected by the addition of DMF in the condensation reaction (Fig. 2b). It is possible that the increase in dielectric constant caused by the addition of DMF ($\varepsilon = 38.2$) to 1-octanol ($\varepsilon = 8.1$) has an effect more important than the increase in ground-state stabilization coming from the higher solubility of *p*-NPG in the presence of DMF, leading to a behavior opposite to what is observed in aqueous media. This would confirm that one (or some) of the enzymes present in commercial preparations is (or are) activated by the presence of DMF, and that this activation does not depend on the presence of a small molecule.

Although the β-glucosidase from almond has been widely studied for "technological" applications [1,2,10–15], it has not been characterized from a molecular point of view, as it has not been cloned or sequenced. It is quite obvious from the results presented here that the high variability in its properties makes the understanding of the behavior of the enzyme quite difficult, and this limits our capacity to design a process based on the use of this enzyme.

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