Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 11, pp. 2213-2216, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0957-4166/94 $7.00+0.00

0957-4166(94)00300-9

Diastereoselective Cleavage of β-Glucosylsulfoxides by β-Glucosidase

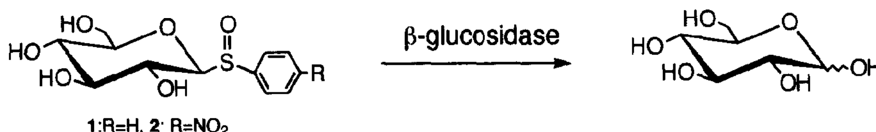
Olaf Karthaus$, Shin-ichiro Shoda, Shiro Kobayashi*

Department of Molecular Chemistry and Engineering, Faculty of Engineering, Tohoku University, Aoba, Sendai 980, Japan

$ new adress: Research Institute for Electronic Science, Hokkaido University, Sapporo 060, Japan

Abstract: Phenyl β-D-glucopyranosyl sulfoxide and *p*-nitrophenyl β-D-glucopyranosyl sulfoxide have been diastereoselectively cleaved by β-glucosidases. Both distereomers can be selectively cleaved, depending on the origin of β-glucosidase.

Enantio- or diastereoselective resolutions of chiral compounds using enzymes has recently become a widespread method in synthetic organic chemistry. For synthetic applications, generally lipases are chosen for the stereoselective cleavage of ester groups.[1] During the screening of new substrates for enzymatic synthesis of oligosaccharides,[2] we found that one of the diastereomers of sulfoxide **1** can be selectively hydrolyzed by β-glucosidase.[3]



This is, the best of our knowledge, the first evidence for the diastereoselective cleavage of sulfoxides by an enzyme. This selective cleavage leads to the synthesis of diastereomerically pure starting materials for many synthetic applications including stereoselective reactions.[4] In 0.1 M acetate buffer (pH 5) without enzyme the sulfoxide **1** is stable. Upon adding a cellulase mixture from *Trichoderma viride*,[5] hydrolysis took place, but only one diastereomer **1a** in the mixture of both phenyl β–glucopyranosyl sulfoxides was cleaved, whereas the other diastereomer **1b** remained unchanged. In order to elucidate which enzyme in the cellulase mixture is responsible for the cleavage reaction, the crude enzyme was fractionated using precipitation in ammonium sulfate solution. This step afforded two fractions, one which shows high β–glucosidase activity, called T.v. A and the other with no activity of β–glucosidase, called T.v. B. Both fractions also contain the other componets of cellulase, endoglucanase and cellobiohydrolase. The T.v. A fraction is still capable of diastereoselective cleavage, whereas T.v. B is no longer able to catalyze hydrolysis (Figure 1a and 1b). The semilogarithmic plots show straight lines for the hydrolysis, indicating a first order reaction, which would be expected in an enzyme-catalyzed hydrolysis at high substrate concentrations.

This finding indicates that β–glucosidase, one enzyme component of the cellulase mixture, is the hydrolase responsible for the diastereoselective cleavage.[6] The selectivity of T.v. A is very high: one

diastereomer **1a** was hydrolyzed completely after 8 h, whereas for the other diastereomer **1b** no cleavage was observed. In order to confirm that β–glucosidase is the active enzyme in the cellulase mixture, enzymes from different origins were checked for the hydrolysis reaction of the sulfoxide. β–Glucosidase from sweet almonds showed significantly different rate in the cleavage of the two diastereomers, but the selectivity was not as high as in the case of β–glucosidase from *Trichoderma viride* (T.v. A), however the same diastereomer **1a** was hydrolyzed preferentially (Figure 1c). β–Glucosidase from *caldocellum saccharolyticum* , a thermophilic archeabacteria, showed no activity on the hydrolysis of **1** (Figure 1d).

Phenyl sulfoxides are known to be good leaving groups under acidic conditions.[7] Therefore, as a control experiment, the enzyme-free hydrolysis was investigated. The substrate **1** was completely stable in buffer at pH 5. Only at very low pH in a strongly acidic solution of triflate (20 % v/v), a slow hydrolysis was observed. In this control experiment diastereoselective cleavage also took place, but the selectivity between the two diastereomers was not as good as in the cases of β–glucosidase from almonds or *Trichoderma viride* (see Table 1).

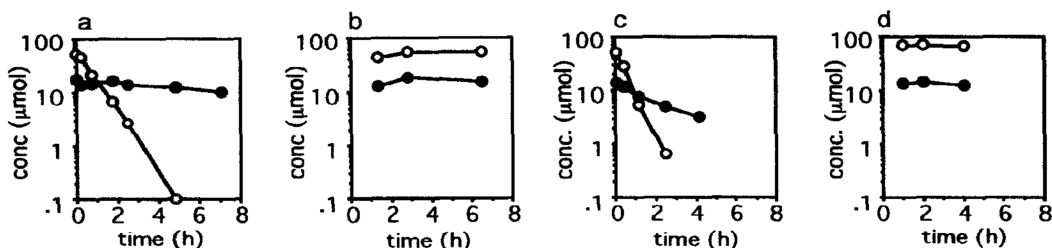


Figure 1: Time conversion curve for the hydrolysis of **1** by β-glucosidase from different origin. a: *Trichoderma viride* (T.v. A), b: *Trichoderma viride* (T.v. B), β–glucosidase (almonds), d: β–glucosidase (*Caldocellum saccharolyticum*). empty circles: **1a**, hashed circles: **1b**

Table 1: Apparent rate constants for the hydrolysis of the two diastereomers of **1** and the ratio between the apparent rate constants of **1a** and **1b**

catalyst	apparent rate constants [h^{-1}]		ratio
	1a	**1b**	
CF_3SO_3H	$1.1x10^{-1}$	$3.7x10^{-2}$	3/1
T.v. A	$5.6x10^{-1}$	$2.6x10^{-2}$	22/1
T.v. B	0	0	
almonds	$7.9x10^{-1}$	$1.5x10^{-1}$	5/1
cald. sacch.	0	0	

Increasing the leaving ability of the sulfoxide by the introduction of a nitro group in the para position of the phenyl ring in **2** led to the expected increase in hydrolysis rate. The selectivity of hydrolysis in the case of the β–glucosidase from *Trichoderma viride* (T.v. A) is still excellent (Figure 2a). Again the glucosidase free fraction of *Trichoderma viride* (T.v. B) showed no activity (Figure 2b). For sweet almonds the hydrolysis was very fast and the apparent rate constant has the same value for both diastereomers (Figure 2c). In contrast to **1**,

the *p*-nitro substituted sulfoxide **2** can be hydrolyzed by β-glucosidase from *Caldocellum saccharolyticum* with high selectivity, but with low rate. Interestingly in this case the other diastereomer **2b** was cleaved preferentially. Hence, this method allows to prepare a diastereomerically pure sample from both diastereomers of glucosyl sulfoxides by choosing the origin of β-glucosidase.

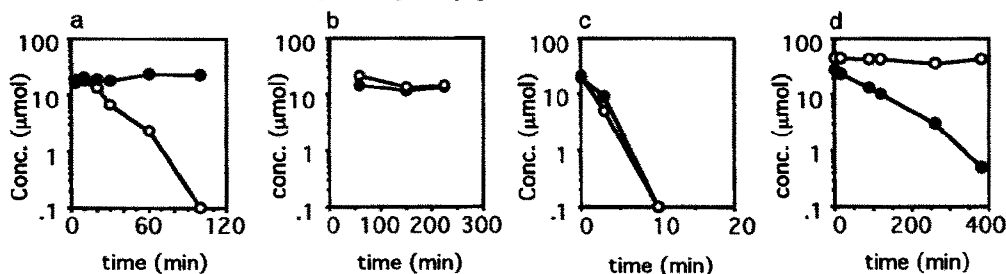


Figure 2: Time conversion curve for the hydrolysis of **2** by β-glucosidase from different origin. a: *Trichoderma viride* (T.v. A), b: *Trichoderma viride* (T.v. B), β-glucosidase (almonds), d: β-glucosidase (*Caldocellum saccharolyticum*). empty circles: **2a**, hashed circles: **2b**

Table 2: Apparent rate constants for the hydrolysis of the two diastereomers of **2** and the ratio between the apparent rate constants of **2a** and **2b**

catalyst	apparent rate constants [h^{-1}]		ratio
	2a	**2b**	
T.v. A	1.38	0	∞
T.v. B	0	0	
almonds	14	14	1/1
Cald. sacch.	4.8×10^{-3}	2.6×10^{-1}	54/1

The present study is the first example of an enzymatic hydrolysis of a glycosyl compound where the chiral group is attached directly to the anomeric center. This should shed light on the mechanism of hydrolysis and the shape of the active site of β-glucosidase.[8] The observed diastereoselectivity can be explained by the following two models: The sulfoxide has a rather fixed conformation and the sterically demanding phenyl group inhibits the enzyme-substrate complex formation for one of the diastereomers. Depending on the origin of the glucosidase, the active site is more or less crowded, leading to the observed differences in the selectivity. In the second model the activation of the sulfoxide is only possible for one diastereomer, depending on the orientaion of the chiral sulfoxide group in the active cavity of the enzyme. Different orientation of the activating amino acid residue and steric demands in the surrounding of the active site will lead to the observed selectivities. The very different rates which were observed in the case of **2** for the different glucosidases can be explained by different strength of the acidic activation of the sulfoxide group. Further attempts to achieve single crystals for a crystal structure analysis of the diastereomerically pure sulfoxides is now in progress.

Experimental:

Cellulase was purified as follows: 500 mg of crude cellulase (Onozuka R-10, *Trichoderma viride*, Yakult Co.) was added to 10 mL of acetate buffer (0.01 M, pH 5.0) and centrifuged at 10000 rpm to remove insoluble material. To the filtrate 3.62 g of $(NH_4)_2SO_4$) was added and the solution was kept at 4°C overnight and filtrated. The filtrate showed no β-glucosidase activity, whereas the residue had high β-glucosidase activity.

The sulfoxides **1** and **2** were synthesized according to Ref 2.[9] In addition CD- and UV spectroscopic data are given for **1a**, the diastereomer which is hydrolyzed by β-glucosidase from *Trichoderma viride* : $\lambda_{ext} = 287$ nm, $\Delta\varepsilon = +5.96°$; $\lambda_{max.} = 292$ nm, $\varepsilon = 7327$ mol^{-1}, CH_3CN, c=0.061 mmol/L. Cellulase Onozuka R-10 from *Trichoderma viride* (activity with carboxymethylcellulose, pH 4.5, 30°C: 6.5 units mg^{-1}) was obtained from Yakult (Japan). β–Glucosidases from almonds (activity with salicin, pH5, 37°C, 5.3 units mg^{-1}) and from *Caldocellum saccharolyticum* (recombinant in *E.coli*, activity with salicin, pH5, 37°C, 30 units g^{-1}) were obtained from Sigma (USA). The activity of β-glucosidase in both *Trichoderma viride* fractions was tested with *p*-nitrophenyl β-D-glucoside.

To 1mL buffer solution (0.1 M acetate buffer, pH 5) of a diastereomeric mixture of **1** or **2** (approx. 0.1 mmol mL^{-1}) was added 1 unit of the enzyme dissolved in 0.5 mL of buffer and the solution was kept at 30°C under shaking. As the reaction proceeds, the clear solution becomes turbid due to the precipitating cleavage product. Aliquots of 0.1 mL were taken and analyzed by HPLC (Hitachi 655 A-12 Liquid Chromatograph (1mL min^{-1}), Hibar RT 250-4 column (25x0.4 cm), filled with Lichrosorb RP 18 (7mm) gel (Merck), Hitachi 655A-30 RI detector, Hitachi D-2000 Chromato Integrator). Both diastereomers have retention times of ca. 40 and 70 min (eluent water), and ca. 10 and 15 min (eluent water : methanol 5:1), respectively. Glucose has retention time of 2.5 min with both solvent systems.

Acknowledgements: OK acknowledges a post doctoral fellowship from the Japan Society for the Promotion of Science and a Feodor v. Lynen scholarship from the Alexander v. Humboldt foundation.

References:

1 J.B. Jones, *Tetrahedron Report* **203**, 3351 (1985)

2a S. Shoda, K. Obata, O. Karthaus, S. Kobayashi, *J. Chem. Soc., Chem. Commun.*, **1993**, 1402

2b O. Karthaus, S. Shoda, H. Takano, K. Obata, S. Kobayashi, *J. Chem. Soc., Perkin 1*, in press

3 This study has been orally presented in parts: O. Karthaus, S. Shoda, S. Kobayashi, *67th Annu. Meet. Chem. Soc. Jpn. Prepr. II*, **1994**, 866.

4 K. Hiroi, *Tohoku Yakka Daigaku Kenkyu Nempo*, **36**, 1 (1989)

5 A.R. White, in "Cellulose and other Natural Polymer Systems" Ed. R.M. Brown, Jr., Plenum, 1982 New York

6 For the substrate specificity of the hydrolysis of β–glucosides with chiral aglycons using β–glucosidase see I.E. Ackermann, D.V. Banthorpe, W.D. Fordham, J.P. Kinder, I. Poots, *J. Plant Physiol.*, **134**, 567 (1989)

7 S. Raghavan, D. Kahne, *J. Am. Chem. Soc.*, **115**, 1580 (1993).

8 H.M. Flowers, N. Sharon, in *Adv. Enzym.*, **48**, Ed. A. Meister, Wiley, New York, 1979

9 The substrate **1** crystallizes as a 1:1 mixture of both diastereomers. The diastereomerically pure samples of **1** and **2** both crystallize as very fine needles.

(*Received 5 September* 1994)