Glycosidic bond rearrangements in isomeric xylobioses by yeast xylan-degrading enzymes

Peter Biely and Eva Petráková

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava, Czechoslovakia

Received 1 October 1984

The cells of *Cryptococcus albidus* induced for xylan-degrading enzymes are capable of transforming 1,2-β-xylobiose and 1,3-β-xylobiose into 1,4-β-xylobiose, the natural inducer. The conversion involves transglycosylation and hydrolysis catalyzed by β-xylosidase and β-xylanase. A probable intermediate of the conversion of 1,2-β-xylobiose was isolated and identified as a trisaccharide, 4-*O*-β-xylopyranosyl-2-*O*-β-xylopyranosyl-D-xylopyranose. The trisaccharide is cleaved by purified endo-1,4-β-xylanase of *C. albidus* mainly at the 1,2-β-linkage yielding xylose and 1,4-β-xylobiose.

Cryptococcus albidus *β-Xylanase* *β-Xylosidase* *β-Xylobiose* *Transglycosylation* *Induction*

1. INTRODUCTION

It is generally accepted that microbial glycanases are induced by the products of their action on polymeric substrates, whereas glycosidases are induced by their substrates [1]. The examples of cellulase induction by sophorse [2–4] and of β-galactosidase induction by allolactose [5,6] suggested that positional isomers of natural products and substrates could serve as better inducers in other systems as well. We have investigated the ability of positional isomers of Xylβ1-4Xyl to induce the xylan-degrading enzyme system in the yeast *Cryptococcus albidus* [7]. Both Xylβ1-2Xyl and Xylβ1-3Xyl were found to serve as inducers, however, the response of the cells to the positional isomers differed considerably from that to Xylβ1-4Xyl. The long induction periods and high enzyme yields obtained after longer incubations in the presence of positional isomers were in contrast to the effects of Xylβ1-4Xyl and thus indicated that the positional isomers may not function as direct inducers, but that they might be first converted to some other active compounds [7]. These observations prompted us to examine the possibility of such transformations in the cells of *C. albidus*.

Abbreviations: Xylβ1-2Xyl, 2-O-β-D-xylopyranosyl-D-xylopyranose; Xylβ1-3Xyl, 3-O-β-D-xylopyranosyl-D-xylopyranose; Xylβ1-4Xyl, 4-O-β-D-xylopyranosyl-D-xylopyranose; Xylβ1-4Xylβ1-4Xyl, xylotriose

2. MATERIALS AND METHODS

2.1. *Chemicals, enzymes and yeast*

Xylβ1-4Xyl was prepared enzymically from phenyl β-D-xylopyranoside [8]. Xylβ1-3Xyl and Xylβ1-2Xyl were prepared synthetically [9,10].

Extracellular endo-1,4-β-xylanase of *C. albidus* was purified as in [11]. A β-xylosidase preparation free of β-xylanase was obtained from a crude hemicellulase of *Aspergillus niger* [12], kindly donated by Dr I.V. Gorbacheva (Bakh Institute of Biochemistry, Academy of Sciences of USSR, Moscow).

Strain *C. albidus* CCY 17-4-1 was grown in a synthetic glucose medium and its xylan-degrading enzyme system was induced with 2 mM methyl β-D-xylopyranoside as in [7].

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

2.2. *Transformation of β-xylobioses by induced cells*

The cells induced with 2 mM methyl β-D-xyloside for 22 h were permeabilized with toluene [11], washed 3 times with cold distilled water to remove low-M_r substances and mixed with 40 mM aqueous solutions of Xylβ1-4Xyl, Xylβ1-3Xyl and Xylβ1-2Xyl, respectively. The concentration of permeabilized cells was about 5 mg/ml (dry wt). The mixtures were incubated under occasional stirring at 30°C, centrifuged at intervals, and aliquots of the supernatants were analyzed by thin-layer chromatography on cellulose in ethyl acetate–acetic acid–water (17:8:10). Reducing sugars were detected with aniline hydrogen phthalate.

The products of 1,2-β-xylobiose (110 mg) treatment were isolated by chromatography on two sheets of Whatman 3MM paper (prewashed with water and dried) in ethyl acetate–acetic acid–water (17:8:7) for 15 h. Precise location of the products was done by means of guide strips and by subsequent detection of the whole band of xylose.

3. RESULTS AND DISCUSSION

Transformation of β-xylobioses under the action of induced and permeabilized cells of *C. albidus* are shown in fig.1. Xylβ1-4Xyl was hydrolyzed to xylose and, at an early stage of the incubation, also converted to Xylβ1-4Xylβ1-4Xyl. Xylβ1-2Xyl was decomposed by the permeabilized cells at about the same rate as Xylβ1-4Xyl. Xylβ1-3Xyl was the poorest substrate for the enzymes present in the cells. In addition to hydrolysis to xylose, both

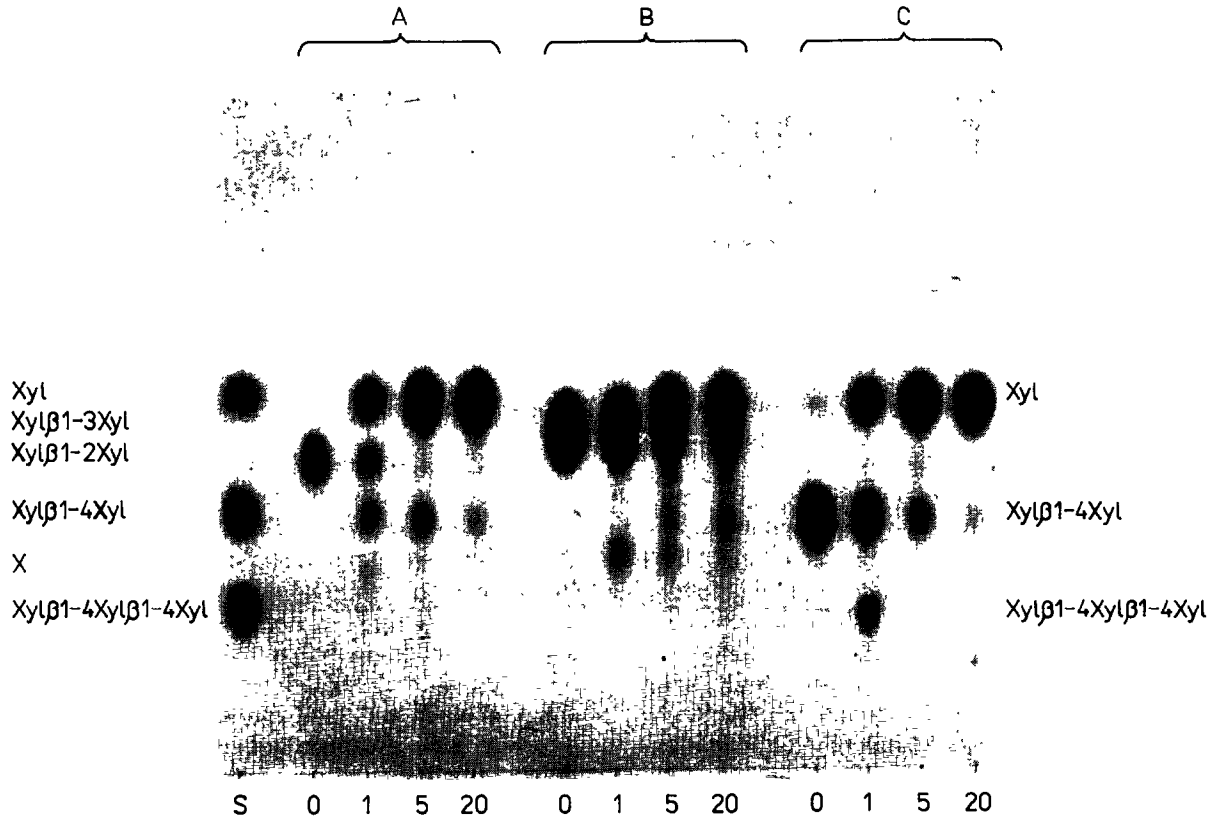


Fig.1. Transformations of β-xylobioses (40 mM) under the action of induced and toluene-permeabilized cells followed by thin-layer chromatography. The cells were incubated at 30°C with 40 mM water solutions of Xylβ1-2Xyl (A), Xylβ1-3Xyl (B) and Xylβ1-4Xyl (C). Incubation time (h) is indicated below. S, standards; X, trisaccharides formed from Xylβ1-2Xyl and Xylβ1-3Xyl.

isomeric xylobioses were converted to several oligosaccharides. Two of them showed chromatographic mobility of Xylβ1-4Xyl and Xylβ1-4Xylβ1-4Xyl. The third oligosaccharide, marked as X, showed chromatographic mobility of a trisaccharide containing at least one glycosidic linkage different from 1,4-β-linkage.

The products formed from Xylβ1-2Xyl were isolated by paper chromatography (table 1). The identity of Xylβ1-4Xyl was confirmed by ^{13}C-NMR spectroscopy [9]. The structure of compound X was established by enzymic hydrolysis and ^{13}C-NMR spectroscopy. The compound (at 20 mM) was hydrolyzed to xylose and Xylβ1-2Xyl by β-xylosidase, and to xylose and Xylβ1-4Xyl by purified β-xylanase (fig.2). Traces of Xylβ1-4Xyl in the mixture with β-xylosidase can be ascribed to transglycosylation reactions taking place at the high substrate concentration. Small amounts of Xylβ1-2Xyl among the products of the β-xylanase digest are due to the formation of an alternative enzyme–substrate complex resulting in the cleavage of the second glycosidic linkage from the reducing end. This observation points to the thus far unknown ability of an endo-1,4-β-xylanase to attack a 1,2-β-xylosidic linkage in a linear xylooligosaccharide.

Definite information on the structure of compound X was obtained from its ^{13}C-NMR spectrum. Its 17 signals were assigned according to the published chemical shifts for all possible

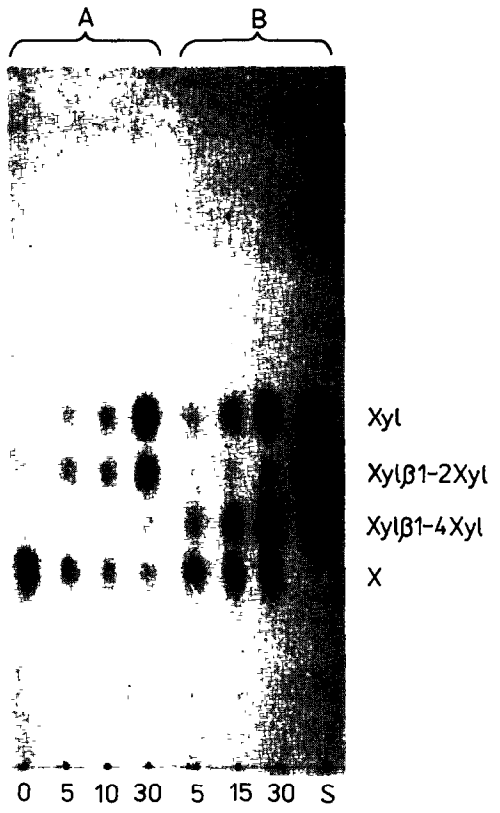


Fig.2. Enzymic hydrolysis of isolated trisaccharide synthesized from Xylβ1-2Xyl in methyl β-D-xyloside-induced cells permeabilized with toluene. Products of hydrolysis by *A. niger* β-xylosidase (A) and *C. albidus* β-xylanase (B). S, standards. Time of incubation (min) is indicated below.

Table 1

Yields and relative chromatographic mobilities of the compounds formed from Xylβ1-2Xyl (110 mg) under the action of induced and permeabilized cells of *C. albidus*

Compound	R_{Xyl}	Yield (mg)
Xylose	1.00	n.d.
Xylβ1-2Xyl	0.73	39.8
Xylβ1-4Xyl	0.58	15.3
Compound X[a]	0.42	12.2
Xylβ1-4Xylβ1-4Xyl	0.33	3.8

[a] Specific rotation of compound X was $[\alpha]_D^{22}$ – 37.2° (*c* 1.4, water)

The mobilities are relative to xylose on Whatman 3MM paper in the system ethyl acetate–acetic acid–water (18:7:8)

xylobioses and their methyl β-glycosides [9] (table 2). According to the spectrum the structure of compound X is identical with the trisaccharide 4-*O*-β-D-xylopyranosyl-2-*O*-β-D-xylopyranosyl-D-xylopyranose (Xylβ1-4Xylβ1-2Xyl). Firm evidence that a 1,2-β-substituted xylose unit is at the reducing end also follows from the doublet of the C-1 signal of the middle xylopyranosyl residue. Such doublets are characteristic for 1,2-β-linked glucobioses and xylobioses [9,13].

Regarding the fact that induced cells of *C. albidus* contain besides β-xylosidase (exo-β-xylanase) also some β-xylanase [11], the conversion of Xylβ1-2Xyl to Xylβ1-4Xyl may be envisaged as a reaction sequence in which both β-xylosidase (E_1) and β-xylanase (E_2) participate, the former enzyme being responsible for the formation

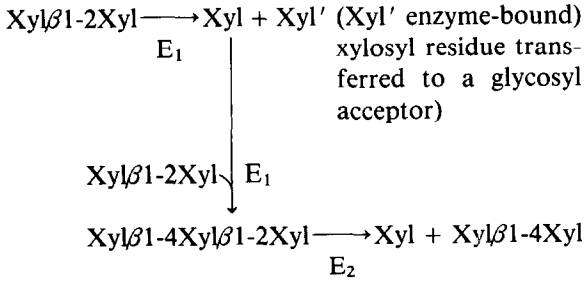
Table 2

^{13}C-NMR chemical shifts of the trisaccharide X formed from Xylβ1-2Xyl under the action of induced permeabilized cells of *C. albidus*

Ring or anomer	Chemical shifts (ppm)				
	C-1	C-2	C-3	C-4	C-5
C-α	93.2	82.0	73.2	70.4	61.8
C-β	96.6	82.9	74.8	70.4	66.5
C'-α	105.8	74.3	76.9	77.7	64.1
C'-β	104.7				
C''	103.0	74.1	76.9	70.4	66.5

C, reducing and xylose unit; C' and C'', non-reducing end units

of the intermediate trisaccharide, and the latter enzyme being responsible for its cleavage at the 1,2-β-linkage:



An analogous mechanism can be proposed for the transformation of Xylβ1-3Xyl.

The above results represent a unique example of glycosidic bond rearrangement taking place in a microbial cell. The results also support the idea that the ability of Xylβ1-2Xyl and Xylβ1-3Xyl to induce the xylan-degrading enzyme system in *C. albidus* is associated with their conversion into Xylβ1-4Xyl, the true inducer structurally related to the respective polysaccharide. Thus the relation of positional isomers of Xylβ1-4Xyl to the induction of the xylan-degrading enzyme system in yeast seems to be completely different from the relation of sophorose to the induction of cellulases in some mycelial fungi [2–4], where cellobiose is believed to serve as a precursor of inducing sophorose. However, methyl β-D-xyloside, an excellent synthetic inducer of the xylan-degrading enzyme system of *C. albidus* [7], was found to be hydrolyzed by induced and permeabilized cells of *C. albidus* only extremely slowly, without any evidence for the formation of xylooligosaccharides by transglycosylations.

ACKNOWLEDGEMENTS

We thank Dr P. Kočiš for measurement of ^{13}C-NMR spectra and Mrs Mária Cziszárová for technical assistance.

REFERENCES

[1] Reese, E.T., Lola, J.E. and Parrish, F.W. (1969) J. Bacteriol. 100, 1151–1154.
[2] Mandels, M., Parrish, F.W. and Reese, E.T. (1962) J. Bacteriol. 83, 400–408.
[3] Nisizawa, T., Suzuki, H., Nakayama, M. and Nisizawa, K. (1971) J. Biochem. (Tokyo) 70, 375–385.
[4] Loewenberg, J.R. and Chapman, C.M. (1977) Arch. Microbiol. 113, 61–64.
[5] Boos, W., Schaedel, P. and Wallenfels, K. (1967) Eur. J. Biochem. 1, 382–394.
[6] Jobe, A. and Bourgeois, S. (1972) J. Mol. Biol. 69, 397–408.
[7] Biely, P. and Petráková, E. (1984) J. Bacteriol. 160, in press.
[8] Krátký, Z., Biely, P. and Vršanská, M. (1981) Carbohydr. Res. 93, 300–303.
[9] Petráková, E. and Kováč, P. (1981) Chem. Zvesti 35, 551–566.
[10] Kováč, P. and Petráková, E. (1980) Chem. Zvesti 34, 537–542.
[11] Biely, P., Vršanská, M. and Krátký, Z. (1980) Eur. J. Biochem. 108, 313–321.
[12] Biely, P. and Vršanská, M. (1983) Carbohydr. Res. 123, 97–107.
[13] Usui, T., Yamaoka, N., Matsuda, K., Tuzimura, K., Sugiyama, H. and Seto, S. (1973) J. Chem. Soc. 2425–2432.