

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

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

*Cryptococcus albidus*       $\beta$ -Xylanase       $\beta$ -Xylosidase       $\beta$ -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 sophorose [2-4] and of  $\beta$ -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 Xyl $\beta$ 1-4Xyl to induce the xylan-degrading enzyme system in the yeast *Cryptococcus albidus* [7]. Both Xyl $\beta$ 1-2Xyl and Xyl $\beta$ 1-3Xyl were found to serve as inducers, however, the response of the cells to the positional isomers differed considerably from that to Xyl $\beta$ 1-4Xyl. 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 Xyl $\beta$ 1-4Xyl 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*.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals, enzymes and yeast

Xyl $\beta$ 1-4Xyl was prepared enzymically from phenyl  $\beta$ -D-xylopyranoside [8]. Xyl $\beta$ 1-3Xyl and Xyl $\beta$ 1-2Xyl were prepared synthetically [9,10].

Extracellular endo-1,4- $\beta$ -xylanase of *C. albidus* was purified as in [11]. A  $\beta$ -xylosidase preparation free of  $\beta$ -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  $\beta$ -D-xylopyranoside as in [7].

**Abbreviations:** Xyl $\beta$ 1-2Xyl, 2-*O*- $\beta$ -D-xylopyranosyl-D-xylopyranose; Xyl $\beta$ 1-3Xyl, 3-*O*- $\beta$ -D-xylopyranosyl-D-xylopyranose; Xyl $\beta$ 1-4Xyl, 4-*O*- $\beta$ -D-xylopyranosyl-D-xylopyranose; Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl, xylotriose

## 2.2. Transformation of $\beta$ -xylobioses by induced cells

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

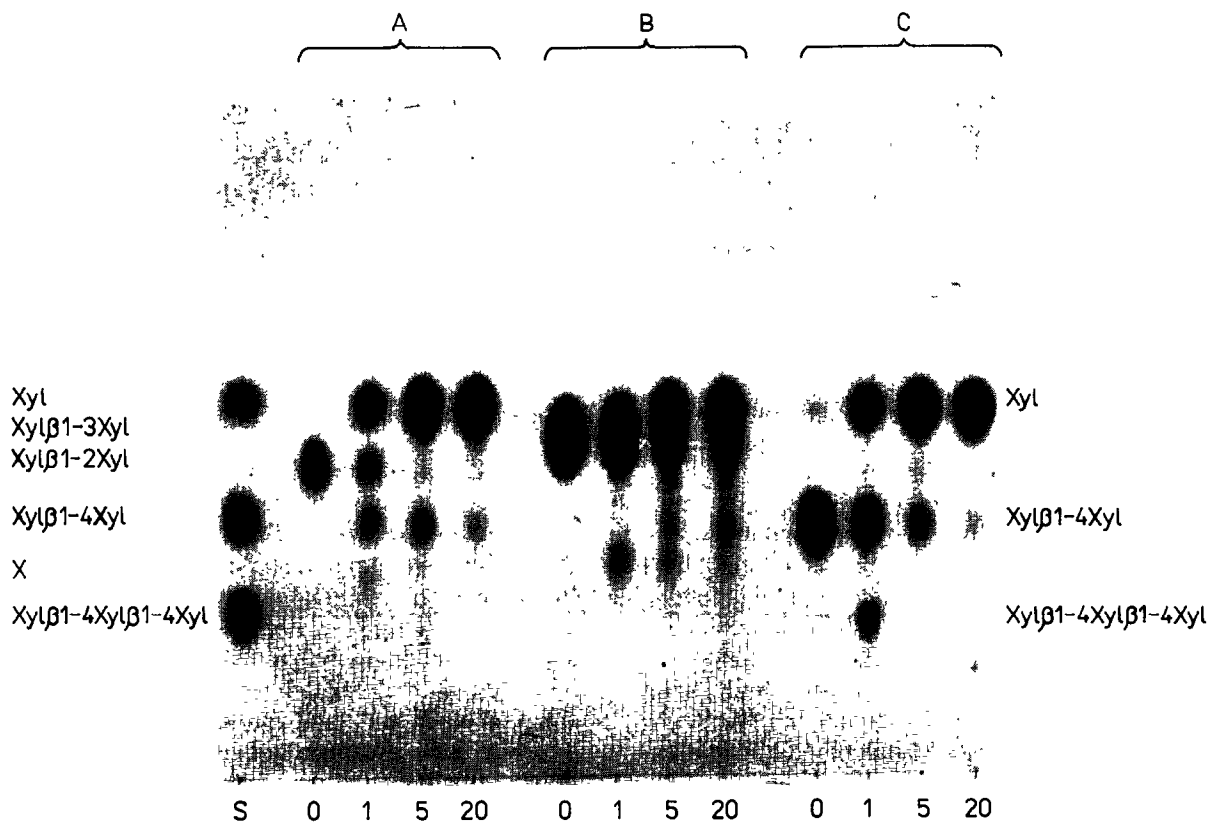


Fig.1. Transformations of  $\beta$ -xylobioses (40 mM) under the action of induced and toluene-permeabilized cells followed by thin-layer chromatography. The cells were incubated at 30°C with 40 mM water solutions of Xyl $\beta$ 1-2Xyl (A), Xyl $\beta$ 1-3Xyl (B) and Xyl $\beta$ 1-4Xyl (C). Incubation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

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

The products formed from Xyl $\beta$ 1-2Xyl were isolated by paper chromatography (table 1). The identity of Xyl $\beta$ 1-4Xyl was confirmed by  $^{13}\text{C}$ -NMR spectroscopy [9]. The structure of compound X was established by enzymic hydrolysis and  $^{13}\text{C}$ -NMR spectroscopy. The compound (at 20 mM) was hydrolyzed to xylose and Xyl $\beta$ 1-2Xyl by  $\beta$ -xylosidase, and to xylose and Xyl $\beta$ 1-4Xyl by purified  $\beta$ -xylanase (fig.2). Traces of Xyl $\beta$ 1-4Xyl in the mixture with  $\beta$ -xylosidase can be ascribed to transglycosylation reactions taking place at the high substrate concentration. Small amounts of Xyl $\beta$ 1-2Xyl among the products of the  $\beta$ -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- $\beta$ -xylanase to attack a 1,2- $\beta$ -xylosidic linkage in a linear xylooligosaccharide.

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

Table 1

Yields and relative chromatographic mobilities of the compounds formed from Xyl $\beta$ 1-2Xyl (110 mg) under the action of induced and permeabilized cells of *C. albidus*

Compound	$R_{\text{Xyl}}$	Yield (mg)
Xylose	1.00	n.d.
Xyl $\beta$ 1-2Xyl	0.73	39.8
Xyl $\beta$ 1-4Xyl	0.58	15.3
Compound X <sup>a</sup>	0.42	12.2
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl	0.33	3.8

<sup>a</sup> Specific rotation of compound X was  $[\alpha]_{\text{D}}^{22} - 37.2^\circ$  (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)

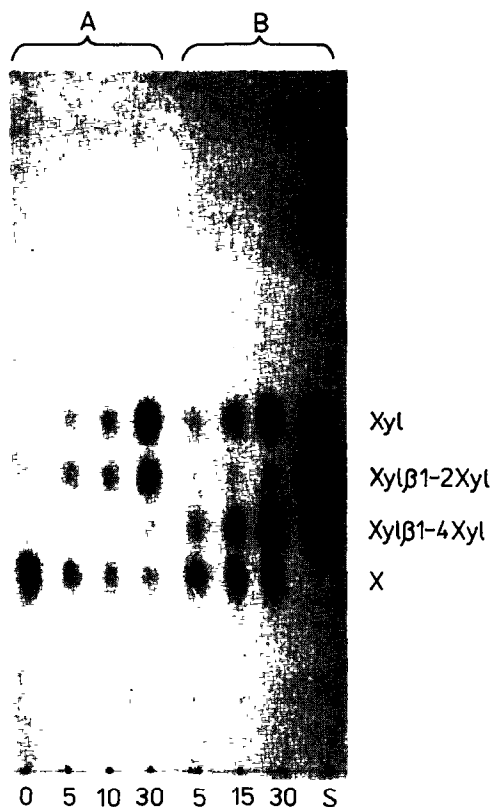


Fig.2. Enzymic hydrolysis of isolated trisaccharide synthesized from Xyl $\beta$ 1-2Xyl in methyl  $\beta$ -D-xyloside-induced cells permeabilized with toluene. Products of hydrolysis by A. *niger*  $\beta$ -xylosidase (A) and *C. albidus*  $\beta$ -xylanase (B). S, standards. Time of incubation (min) is indicated below.

xylobioses and their methyl  $\beta$ -glycosides [9] (table 2). According to the spectrum the structure of compound X is identical with the trisaccharide 4-O- $\beta$ -D-xylopyranosyl-2-O- $\beta$ -D-xylopyranosyl-D-xylopyranose (Xyl $\beta$ 1-4Xyl $\beta$ 1-2Xyl). Firm evidence that a 1,2- $\beta$ -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- $\beta$ -linked glucobioses and xylobioses [9,13].

Regarding the fact that induced cells of *C. albidus* contain besides  $\beta$ -xylosidase (exo- $\beta$ -xylanase) also some  $\beta$ -xylanase [11], the conversion of Xyl $\beta$ 1-2Xyl to Xyl $\beta$ 1-4Xyl may be envisaged as a reaction sequence in which both  $\beta$ -xylosidase ( $E_1$ ) and  $\beta$ -xylanase ( $E_2$ ) participate, the former enzyme being responsible for the formation

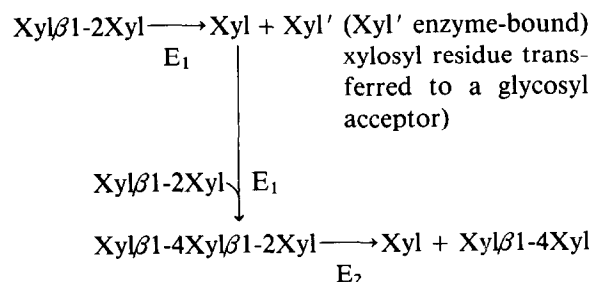
Table 2

$^{13}\text{C}$ -NMR chemical shifts of the trisaccharide X formed from Xyl $\beta$ 1-2Xyl 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- $\alpha$	93.2	82.0	73.2	70.4	61.8
C- $\beta$	96.6	82.9	74.8	70.4	66.5
C'- $\alpha$	105.8	74.3	76.9	77.7	64.1
C'- $\beta$	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- $\beta$ -linkage:



An analogous mechanism can be proposed for the transformation of Xyl $\beta$ 1-3Xyl.

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 Xyl $\beta$ 1-2Xyl and Xyl $\beta$ 1-3Xyl to induce the xylan-degrading enzyme system in *C. albidus* is associated with their conversion into Xyl $\beta$ 1-4Xyl, the true inducer structurally related to the respective polysaccharide. Thus the relation of positional isomers of Xyl $\beta$ 1-4Xyl 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  $\beta$ -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.

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