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DETERMINATION OF THE ANOMERIC CONFIGURATION OF D-XYLOSE WITH D-XYLOSE ISOMERASES

HILDA KERSTERS-HILDERSON, MARC CLAEYSSENS, ELISA VAN DOORSLAER, AND CLEMENT K. DE BRUYNE

Laboratorium voor Algemene en Biologische Scheikunde, Rijksuniversiteit Gent, B9000 Gent (Belgium)

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ABSTRACT

The anomeric configuration of D-xylose, resulting from hydrolysis of β-D xylopyranosides by β-D-xylosidase from *Bacillus pumilus*, has been determined by an enzymic procedure, based on the stereospecificity of D-xylose isomerases. The initial hydrolysis product is α-D-xylose. β-D-Xylosidase from *Bacillus pumilus* thus acts by inversion of configuration in contrast to most other glycosidases.

INTRODUCTION

Elucidation of the anomeric configuration of the initial hydrolysis product from oligosaccharides or glycosides by carbohydrases is of considerable importance from the mechanistic viewpoint. Following Koshland's interpretation[1], inversion of configuration is explained in terms of a "single displacement" mechanism, whereas retention of configuration should correspond to a "double displacement" mechanism. According to Reese's classification[2], retention of configuration occurs with glycosidases, whereas inversion of configuration would be more characteristic of exoglycanases.

O.r.d., g.l.c., and n.m.r. spectroscopy can be used to determine the anomeric nature of sugars as initial hydrolysis products. An enzymic method, using β-stereospecific D-glucose oxidase[3] for determination of the anomeric nature of enzymically liberated D-glucose, has also been reported[4]. The latter method is of particular interest, as neither specific instrumentation nor large amounts of enzyme or substrates are required.

This paper deals with another enzymic assay, which is specific for determining the anomeric nature of the D-xylose initially formed on hydrolysis by xylosidase. The α-stereospecificity of D-xylose isomerases, as shown[5–7] for a *Streptomyces* sp., has been confirmed for the D-xylose isomerase from *Lactobacillus brevis*. Its suitability for elucidating the anomeric nature of enzymically liberated D-xylose has been established.

RESULTS AND DISCUSSION

Anomeric specificity of Lactobacillus brevis D-*xylose isomerase.* — An accurate and rapid method for assaying D-xylose isomerase was recently developed by Yamanaka, and is based on a coupled reaction with D-glucitol dehydrogenase[8]; the latter enzyme also possesses dehydrogenase activity for xylitol and ribitol. We have used a similar procedure to investigate the anomeric specificity of the D-xylose isomerase from *Lactobacillus brevis*. The xylose concentrations were below the K_m value[8–9].

In one experiment, 200 µl of 3mM α-D-xylose, freshly dissolved in 0.05M phosphate buffer (pH 7), was incubated with 5 µl of a solution of D-xylose isomerase (0.5 unit) and 10 µl of 0.03M $MnCl_2$ for 1 min at 30°. After 1 min, 0.8 ml of twice-distilled water, 10 µl of 0.1M glutathione, and 10 µl of 0.01M NADH were added, and the oxidation of NADH was started by the addition of 10 µl of a solution of D-glucitol dehydrogenase (0.17 unit).

In an alternative experiment, similar conditions were chosen except that a mutarotated solution of D-xylose was used, corresponding to 32.1–34.8% of α-D form[10].

As illustrated in Fig. 1(*a*), the absorbance at 340 nm decreased rapidly when freshly dissolved α-D-xylose was used as substrate. However, with the mutarotated solution [Fig. 1(*b*)], corresponding to only 32.1–34.8% of α-D-xylose, NADH was oxidized at a much lower rate, which indicates that α-D-xylose is the preferred substrate

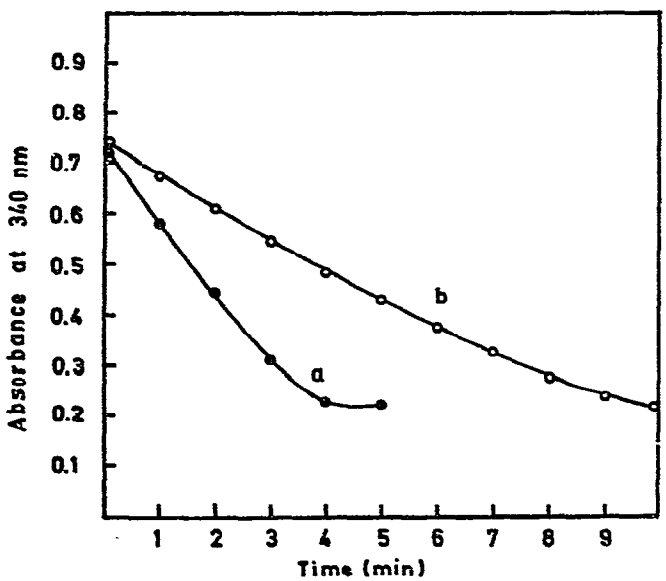

Fig. 1. Time dependence of the oxidation of NADH for the D-xylose isomerase–D-glucitol dehydrogenase system: (*a*) freshly dissolved α-D-xylose (—●—); (*b*) equilibrated solution of α- and β-D-xylose, corresponding to 32.1–34.8% of α-D form[10] (—○—).

for the D-xylose isomerase from *Lactobacillus brevis*. This α-stereospecificity agrees with the generalized view of Wurster *et al.*[5], and with the results of Schray *et al.*[6] and Feather *et al.*[7] for the D-xylose isomerase of *Streptomyces* sp.

Anomeric form of D-*xylose as reaction product of the hydrolysis of aryl* β-D-*xylopyranosides by* β-D-*xylosidase from* Bacillus pumilus. — In a similar set of experiments, the anomeric configuration of the enzymically liberated D-xylose was determined with D-xylose isomerase from *Lactobacillus brevis*. The conditions selected were such that hydrolysis of the substrate *p*-cyanophenyl β-D-xylopyranoside[11] was complete in one minute, and that the xylose concentration produced by enzymic hydrolysis corresponded to the xylose concentration of the experiments described above (see Fig. 1).

The reaction mixture, containing 100 µl of a solution of β-D-xylosidase (0.8 unit), 5 µl of a solution of D-xylose isomerase (0.5 unit), 10 µl of 0.03M $MnCl_2$, and 100 µl of 6mM *p*-cyanophenyl β-D-xylopyranoside in phosphate buffer (0.05M, pH 7), was incubated at 30° for 1 min. After 1 min, 0.8 ml of twice-distilled water, 10 µl of 0.1M glutathione, and 10 µl of 0.01M NADH were added, and the oxidation of NADH was started by the addition of 10 µl of a solution of D-glucitol dehydrogenase (0.17 unit).

In a second experiment, β-D-xylosidase and its substrate were incubated for 15 min, in the absence of D-xylose isomerase. Since the mutarotation of D-xylose is

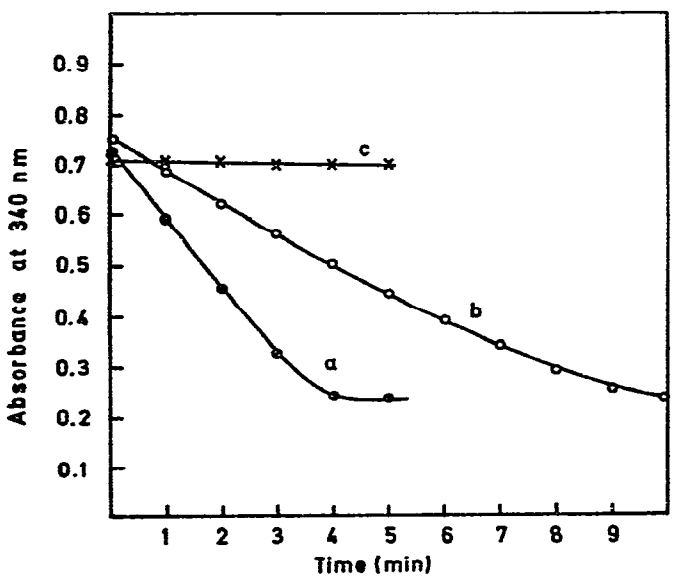

Fig. 2. Time dependence of the oxidation of NADH for the coupled system of β-D-xylosidase–D-xylose isomerase and D-glucitol dehydrogenase: (*a*) D-xylose isomerase and β-D-xylosidase added simultaneously to the aryl β-D-xylopyranoside solution (—●—); (*b*) D-xylose isomerase added 15 min after the hydrolysis was started, to enable mutarotation of the liberated D-xylose (—○—); (*c*) blank experiment without substrate (—×—).

fast[10], an equilibrium solution (32.1–34.8% α-form) of the enzymically liberated D-xylose was obtained under these conditions. After 15 min, the reaction mixture was incubated with D-xylose isomerase for 1 min, twice-distilled water, glutathione, NADH, and D-glucitol dehydrogenase were added as described above, and the NADH oxidation was followed at 340 nm. Total hydrolysis of the aryl xyloside was confirmed by assay of D-xylose[12] on aliquot samples (20 μl) from a β-D-xylosidase–substrate mixture which did not contain D-xylose isomerase, after hydrolysis for 1 and 15 min.

As depicted in Fig. 2(*a*), a typical response for α-D-xylose is obtained when β-D-xylosidase and D-xylose isomerase were added simultaneously [*cf.* Fig. 1(*a*)]. In contrast, the rate of NADH oxidation is only 47% in the second experiment [Fig. 2(*b*)] where mutarotation was possible [*cf.* Fig. 1(*b*)]. Since D-xylose isomerase is specific for α-D-xylose, the decrease in oxidation rate of NADH is clearly due to the smaller amount of α-D-xylose present in the mutarotated sample and strongly suggests that the anomeric configuration of the liberated D-xylose is α. This conclusion indicates that inversion of configuration takes place during the hydrolysis of aryl β-D-xylopyranosides by β-D-xylosidase from *Bacillus pumilus*. Analogous, qualitative and quantitative results were obtained when *p*-iodophenyl β-D-xylopyranoside was used as substrate.

Considering Reese's criteria[2], one can raise the question of the enzyme's being a real xylosidase or an exo-xylanase.

Work is in progress to elucidate this problem, and the coupled-assay system, described here, is being further developed for quantitative measurements of D-xylose liberated enzymically from xylo-oligosaccharides.

EXPERIMENTAL

α-D-Xylose (>95%) was a commercial product (Merck). *p*-Substituted aryl β-D-xylopyranosides were prepared by a procedure described previously[13]. L-Glutathione (>98%, Fluka) and NADH (disodium salt, Cal. Biochem.) were commercial products. Buffer materials were analytical grade and made up with water distilled twice from quartz.

β-D-Xylosidase [β-D-xyloside xylohydrolase (EC 3.2.1.37)] from *Bacillus pumilus* was isolated and purified as described previously[14–15]. Routine enzymic assays were performed[14] with 4mM *p*-nitrophenyl β-D-xylopyranoside by measuring the increase in absorbance at 400 nm[14]. With other substrates, the hydrolysis was followed discontinuously, measuring either D-xylose[12] or phenol[16–17]. One unit of β-D-xylosidase activity was defined as reported earlier[18].

D-Xylose isomerase [D-xylose ketol-isomerase (EC 5.3.1.5)] from *Lactobacillus brevis*[9] was a generous gift from Professor K. Yamanaka, and D-glucitol dehydrogenase [L-iditol:NAD oxidoreductase (EC 1.1.14)] from sheep liver was purchased from Boehringer. D-Xylose isomerase was assayed spectrophotometrically as NADH oxidation in a coupled reaction with D-glucitol dehydrogenase[8]. Enzyme units are defined as previously described[8,9].

All assays were carried out with a Beckman DBG-T spectrophotometer, thermostated at 30° and equipped with a log Ten-Inch recorder.

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