D-XYLOSE METABOLISM BY CELL-FREE EXTRACTS OF *PENICILLIUM CHRYSOGENUM*

CHING CHIANG AND S. G. KNIGHT

Department of Bacteriology, University of Wisconsin, Madison, Wisc. (U.S.A.)

(Received December 10th, 1958)

SUMMARY

The initial steps of D-xylose metabolism by a cell-free extract of *Penicillium chrysogenum* involve the reduction of xylose to xylitol by TPNH, followed by DPN-linked oxidation of xylitol to xylulose. The enzymically formed products, xylitol and xylulose, have been isolated and identified.

The enzyme which catalyzes the reduction of xylose to xylitol and is tentatively named D-xylose reductase, was purified 20-fold over the crude extract; it is an inducible enzyme and relatively specific to D-xylose. The K_s for D-xylose is 0.09 M and for TPNH, $2.2 \cdot 10^{-5}$ M. The enzyme had approximately maximum activity at pH 7.2. Fluoride at 0.05 M and zinc at 0.001 M were strong inhibitors. The inhibition by *p*-chloromercuribenzoic acid could be restored by glutathione or cysteine. There was no effect by arsenite, cyanide, iodoacetic acid and Versene at 0.01 M, as well as magnesium, calcium, manganese, and dipicolinic acid at 0.001 M.

INTRODUCTION

In an early report[1], evidence was presented that the initial step in the utilization of D-xylose by cell-free extracts of *Penicillium chrysogenum* was a reduction of D-xylose to D-xylitol. The enzyme responsible for this reaction has a specific requirement for TPNH* as the coenzyme, and the equimolar conversion by the enzyme indicates a one-step reaction. The enzymically produced D-xylitol was isolated and identified tentatively. This paper describes the partial purification and certain properties of this enzyme, which has been named D-xylose reductase, and further observations in which the D-xylitol is reoxidized to D-xylulose by a DPN-linked enzyme in the crude cell-free extract.

METHODS AND MATERIALS

Preparation of the crude enzyme extract

Penicillin chrysogenum strain NRRL 1951-B25 from the laboratory stock was maintained in soil stock culture. Soil from the stock was sprinkled over the slanted

* Abbreviations: ATP, adenosine triphosphate; DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide; Tris, tris (hydroxymethyl) aminomethane.

References p. 463.

agar surface of a synthetic medium in 6-ounce prescription bottles; the synthetic medium consisted of 1 % D-xylose, 0.5 % NH_4NO_3, 0.05 % $MgSO_4$, 0.01 % $FeSO_4$, as well as 0.001 % each of $MnSO_4$, $CaCl_2$, and $ZnCl_2$; 1.5 % agar was added and the final pH was adjusted to 7.2 by adding 0.2 % K_2HPO_4. The bottles were incubated for 4 days at 25°; after which 15 ml of sterile distilled water were added and bottles shaken to obtain a uniform spore suspension. A 1-ml aliquot of the spore suspension was inoculated into a 250-ml Erlenmeyer flask containing 50 ml of the synthetic medium with 2 % D-xylose which had been sterilized separately. The inoculated flask was incubated at 30° for 24 h on a shaker with a 3/4-inch radius and a speed of 355 cyc./min. This culture then served as the final inoculum; a 5-ml aliquot was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of the 1 % D-xylose-synthetic medium. These flasks were incubated under the same conditions for another 48 h. The mycelia were harvested by filtering through a suction funnel and washed with about 7 l of cold 0.01 *M* phosphate buffer, pH 7.2, per l of culture. The crude enzyme extract was prepared by grinding the wet mycelia with an equal weight of acid-washed sand in a chilled mortar for 10–25 min. 3 to 4 vol. of 0.01 *M* phosphate buffer, pH 7.2, was then added and the cellular debris and the sand were removed by centrifugation for 10 min at 2590 × *g* in a Servall centrifuge. The supernatant containing about 5–7 mg of protein/ml was used as the source of enzyme; it was stored at 4° in the refrigerator.

Partial purification of the enzyme D-*xylose reductase*

All operations were performed at 0–4°. Precipitations were separated by centrifugation at 10,000 × *g* in a Servall centrifuge for 10 min. 100 ml of crude cell free extract, in 0.1 *M* phosphate, pH 7.2 and containing 7.2 mg protein/ml, was adjusted to pH 6.2 by adding 1 *M* acetic acid; then 50 ml of calcium phosphate gel (2.9 dry wt.) was added in a protein to gel ratio 1:4 and the mixture was stirred for 5 min. The gel was removed by centrifugation, washed once with 10 ml of 0.1 *M* phosphate buffer, pH 6.2, and discarded. The supernatant and wash were combined. This treatment removed the enzymes which endogenously reduced TPN. The solution containing the enzyme was stirred continuously and 36.9 g of finely powdered $(NH_4)_2SO_4$ was added to give 0.34 saturation and allowed to stand for 20 min. The precipitate was removed and discarded. The supernatant was treated again in the same manner

TABLE I

PURIFICATION OF D-XYLOSE REDUCTASE

Fraction	*Total vol (ml)*	*Units/ml*	*Total units*	*Protein (mg/ml)*	*Total protein*	*Specific activity*	*Recovery %*
Crude extract	100	260	26,000	7.2	720	36	100
1st calcium phosphate gel supernatant	143	180	25,800	1.2	172	150	99
1st ammonium sulfate precipitate (0.34–0.58)	8	2,240	17,900	8.3	66.4	270	69
2nd calcium phosphate gel supernatant	10	1,440	14,400	4.0	40	360	55
2nd ammonium sulfate precipitate (0.40–0.50)	5.4	2,400	12,900	3.2	17.3	750	50

References p. 463.

with an additional 26.2 g of $(NH_4)_2SO_4$ to obtain 0.58 saturation and allowed to settle for another 30 min. Then, the precipitate was recovered and mixed into 0.02 *M* phosphate buffer, pH 7.0, which further removed insoluble substances; the enzyme solution now was devoid of the ability to endogenously oxidize TPNH. The solution was then adjusted to pH 5.5 with 1 *M* acetic acid and treated with 0.133 g of calcium phosphate gel in a protein to gel ratio 1:2. After being stirred or 2–3 min, the gel was removed and washed twice with 0.5 ml of 0.1 *M* acetate buffer, pH 5.5, and then discarded. The combined solution of supernatant and washings was adjusted to 0.4 saturation with concentrated $(NH_4)_2SO_4$ (saturated at 0° and warmed up to 4°). After standing for 15 min, the precipitate was removed, and the supernatant was further treated to 0.5 saturation, and allowed to stand for another 15 min. The precipitate was recovered and dissolved in 0.1 *M* phosphate buffer, pH 7.2. This enzyme preparation, which was purified 20-fold over the crude extract (Table I) and had a value of 1.24 for the optical density ratio at 280:260 mμ, was used to determine the properties of the enzyme.

Assay of D-*xylose reductase*

The activity of D-xylose reductase was determined spectrophotometrically as the decrease in optical density at 340 mμ when TPNH was oxidized in the presence of D-xylose. To a 1-cm cell with a 1-cm light path, was added 0.1 ml of $1.15 \cdot 10^{-3}$ *M* TPNH in 0.1 *M* tris buffer, pH 7.5; 0.1 ml of enzyme in a suitable dilution in 0.01 *M* phosphate buffer, pH 7.0, to obtain 10 to 60 units of enzyme/ml; 0.1 ml of water or solution to be tested; and 0.6 ml of 0.1 *M* phosphate or tris buffer, pH 7.0. This mixture was allowed to equilibrate for 1 min at 25°, then readings were taken at 340 mμ at 15-second intervals after mixing with 0.1 ml of 0.1 *M* D-xylose. One unit is defined as that amount of enzyme which caused an initial rate of change in optical density of 0.010/min under the above conditions. Specific activity is expressed as units/mg of protein. In this assay the velocity was proportional to the enzyme concentration up to 7 units of D-xylose reductase/ml of reaction mixture. This assay method was useful also when applied to the crude extract; the 1-min equilibration of the reaction mixture before addition of the substrate allowed complete endogenous oxidation of TPNH. The rate of endogenous reduction of TPN in the crude extract was corrected with the rate obtained from the incubation mixture using TPN instead of TPNH.

Analytical methods

Spectrophotometric measurements were made with either a Beckman spectrophotometer model DU or DK-2 recording spectrophotometer in 3.0 or 1.0 ml cells (l = 1 cm). The melting point was determined with a Fisher-Jones melting point apparatus. D-xylulose was determined by the method of DISCHE AND BORENFREUND[2] with a 2-h incubation at 37° and with D-xylulose monoacetone as the standard. The orcinol reaction was done by the modification of HORECKER *et al.*[3]. The determination of protein was made by the method of LOWRY *et al.*[4]. Separation of free sugars was accomplished with a Dowex 1-borate column[5].

Chemicals

D- and L-xylulose and D-xylulose monoacetone were the gift of Dr. W. A. WOOD.

References p. 463.

TPNH, DPNH (both 90% purity) and lactic dehydrogenase were purchased from the Sigma Chemical Company; TPN and DPN were from the Pabst Laboratories. All other chemicals were from various commercial sources.

EXPERIMENTAL

Experiments with crude enzyme preparations

Reduction of D-*xylose:* The crude enzyme preparation oxidized TPNH, but not DPNH, with D-xylose as the substrate. Under the same conditions, DL-glyceraldehyde, D-erythrose, L-arabinose, and D-ribose also served as substrates for the oxidation of TPNH. The following sugars did not show demonstrable rates of TPNH oxidation: L-xylose, D-arabinose, D-glucose, D-galactose, D-mannose, D-rhamnose, D-gluconic acid and D-galacturonic acid. When cells were grown on synthetic medium with glucose as the sole carbon source, the cell-free preparation could not oxidize TPNH, or DPNH, with any of the above aldopentoses or aldohexoses as substrate but did oxidize TPNH in the presence of either DL-glyceraldehyde or D-erythrose. All attempts to demonstrate a D-xylose isomerase or a xylokinase were unsuccessful.

The enzymically formed D-xylitol was isolated and identified tentatively by paper chromatography, as reported in the preliminary communication. An attempt was made to further characterize this compound by converting it to a pentaacetate derivative as follows: To 23 mg of the syrup, 0.3 ml acetic anhydride and 0.3 ml pyridine were added, and the reaction mixture was shaken mechanically at 25° overnight. Then, 3.5 ml of water at 4° was added to this mixture but no precipitate formed at this time. The derivative precipitated after refrigeration for 2 days; precipitation could be accelerated by occasional vigorous stirring. The precipitate was collected by filtration, washed 3 times with small portions of cold water, and dried over $CaCl_2$ *in vacuo*; 14.2 mg of very fine, white amorphous powder were obtained. This powder was dissolved in a small amount of ether and allowed to crystallize at 4° in the refrigerator. After approximately 12-h, the crystal formed as a large, clear, tabular prism; it was collected by filtration, washed with ether, dried over $CaCl_2$ *in vacuo*; and weighed 12.2 mg. This crystal melted at 60–61°, which was the melting point of the authentic D-xylitol pentaacetate prepared by the same procedure; here, however, an amorphous precipitate came down shortly after adding the cold water to the reaction mixture. No significant depression in mixed melting point (59.5–61°) was observed.

Oxidation of D-*xylitol to* D-*xylulose*

The D-xylitol formed is an intermediate in the initial utilization of D-xylose and is subject to further metabolism by the cell-free extract. An enzyme which is specific for DPN as the hydrogen acceptor is responsible for the oxidation of D-xylitol. The addition of Mg^{++} stimulated the rate of the oxidation. The enzymic activity varied in different batches of crude enzyme preparation; the variation probably resulted from varying degrees of cell breakage. Fig. 1 illustrates D-xylitol oxidation measured as the reduction of DPN. TPN was reduced also by the extract but with no difference in rate in the presence or absence of D-xylitol. Accordingly from these results, it was concluded that D-xylitol was not oxidized reversibly to D-xylose with TPN under these conditions.

References p. 463.

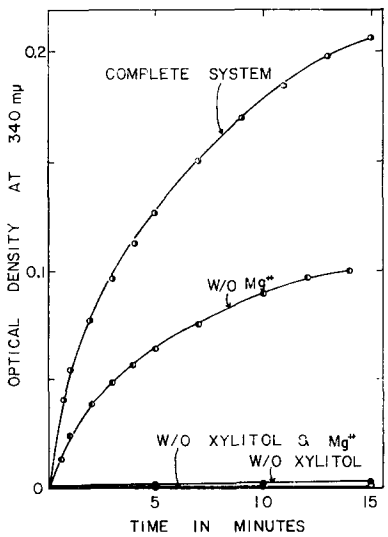

Fig. 1. The DPN-linked oxidation of xylitol. The complete system contained 40 μmoles D-xylitol, 0.9 μmoles DPN, 10 μmoles $MgCl_2$, 200 μmoles tris(hydroxymethyl)aminomethane buffer, pH 7.5, and 0.5 ml crude cell-free extract, in a total volume of 2.5 ml. Incubation was at room temperature. The reaction was initiated by adding the xylitol.

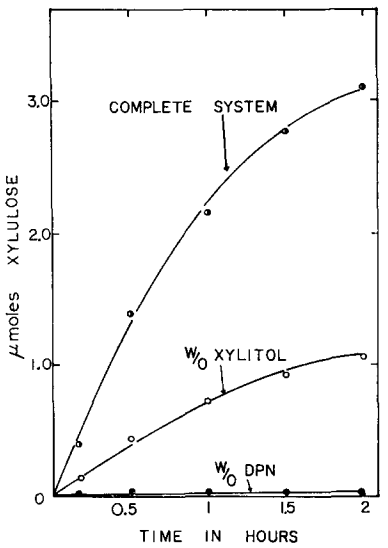

Fig. 2. The formation of xylulose from xylitol. The reaction mixture contained 320 μmoles tris-(hydroxymethyl)-aminomethane buffer, pH 7.,5 20 μmoles D-xylitol, 25 μmoles DPN, 10 μmoles $MgCl_2$, and 2.5 ml cell-free extract in a total volume of 6.5 ml. All reactions were incubated at 30°. A 1-ml aliquot was removed at the designated time and precipitated with 2 ml of 5% $ZnSO_4$ and 2 ml of 0.3 *N* $Ba(OH)_2$, the reagents having been adjusted to pH 7.2. The mixture was further treated with charcoal and the supernatant was analyzed for xylulose.

The kinetics for the formation of a ketopentose from the oxidation of D-xylitol was determined next. In a complete system containing Tris buffer at pH 7.5, Mg^{++} and a xylitol to DPN ratio of 1:1.25, the yield of ketopentose was 15% after 2-h incubation (Fig. 2). No increase in yield was obtained by trapping with borate at pH 8.0. A slight increase to 20% in ketopentose production was obtained by coupling with the lactic dehydrogenase–pyruvate system to remove the DPNH. The ketopentose was identified as xylulose by the characteristic color produced in the cysteine–carbazole reaction; the color development required over 15 min. Furthermore, the enzymically produced xylulose showed an identical absorption spectrum to authentic xylulose in the orcinal reaction after being purified on a Dowex-1-borate column (Fig. 3).

According to the symmetrical configuration of the xylitol molecule, the xylulose formed from enzymic oxidation with DPN may be in the D- or L-form, or a mixture of both, depending upon whether the xylitol molecule is oxidized on the C-2 or C-4 position. By assuming the oxidation to be reversible, the specificity of the isomers could be determined in an incubation mixture with DPNH and D- or L-xylulose. Fig. 4 shows a rapid decrease in absorption in the DPNH–D-xylulose mixture and very little decrease in absorption with L-xylulose. Thus, the oxidation product of D-xylitol is D-xylulose and also the equilibrium of this reaction favors the reduction of D-xylulose.

References p. 463.

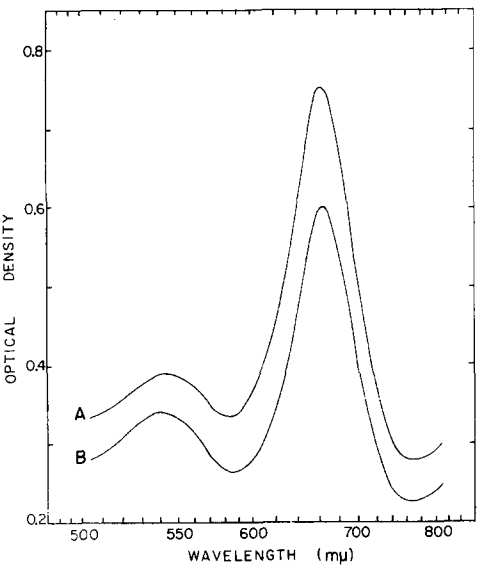

Fig. 3. The absorption spectra of the xylulose-orcinal reaction products. (A) authentic xylulose; (B) oxidation product of xylitol purified on Dowex 1-borate column. The xylitol was obtained by the following procedure: a mixture containing 40 μmoles D-xylitol, 50 μmoles sodium pyruvate, 10 mg DPN, 2 mg lactic acid dehydrogenase, 20 μmoles $MgCl_2$, 1 mmole tris buffer, pH 7.5, and 5 ml crude cell-free extract in a total volume of 10 ml, was incubated at 30° for 5 h. The aliquot was deproteinized by heating in boiling water for 5 min, followed by centrifugation and deionization by passing through Amberlite IR-120(H^+) and IR(OH^-) columns. After evaporation to dryness, the concentrated syrup was taken up in 10 ml 0.005 *M* $K_2B_4O_7 \cdot 4H_2O$. The xylulose came off in the first peak from this column (fraction 68–71), in a total amount of 8.7 μmoles.

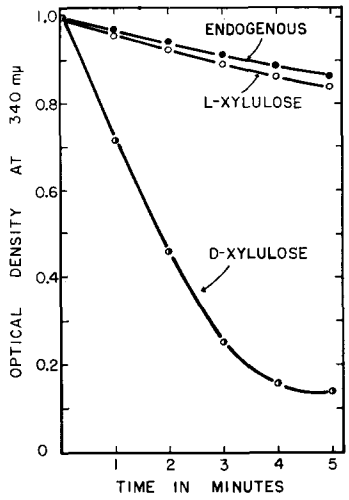

Fig. 4. Oxidation of DPNH by D- and L-xylulose. The reaction mixture contained 0.16 μmoles DPNH, 2.5 μmoles either D- or L-xylulose, 80 μmoles tris(hydroxymethyl)aminomethane buffer, pH 7.5, and 0.1 ml crude cell-free extract, in a total volume of 1.0 ml. Incubation was at room temperature. The reaction was initiated by adding DPNH.

Properties of partially purified D-*xylose reductase*

Stability: The enzyme preparation purified 20-fold had no loss in activity after having been stored at 4° for 3 months, but was destroyed completely when frozen to —12° overnight. There was no significant loss in activity when the enzyme was diluted 8 fold in 0.01 *M* phosphate buffer, pH 7.0 and held at 0° for 5 h. The effect of pH on the storage stability of the enzyme was tested; there was a symmetrical declination of activity on both sides of pH 7.0 when the enzyme preparations were held at 0° and there was somewhat more rapid inactivation at 30° (Fig. 5).

Effect of pH and buffer: There was no difference in activity when enzymic reactions were carried out in 0.1 *M* Tris or phosphate buffer at pH 7.0. The enzyme had no sharp optimum pH but had approximately maximum activity near pH 7.2 (Fig. 6).

Activators and inhibitors: The enzyme was not stimulated by glutathione or cysteine; however, these two compounds restored activity after the enzyme had been inactivated by *p*-chloromercuribenzoic acid (Table II). Other enzyme poisons, arsenite, cyanide, and iodoacetic acid, at a final concentration of $1 \cdot 10^{-2}$ *M*, did not decrease the activity, but fluoride inhibited slightly and inhibition was increased

References p. 463.

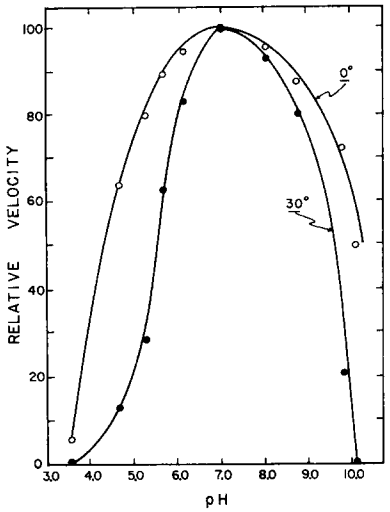

Fig. 5. The effect of pH and temperature on the stability of D-xylose reductase. The buffer systems at 0.1 *M* were: pH 3.0–5.0, citrate–phosphate; pH 5.0–7.0, phosphate; pH 7.0–9.0, tris–HCl; and above pH 9.0, glycine–NaOH. Enzyme was diluted from the 20-fold purified preparation to 1 mg protein/ml with the above buffer and held at the designated temperature for 2 h. The assay condition was the same as described and 6 units of protein were used.

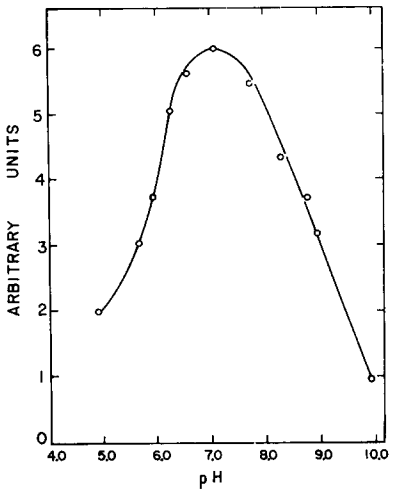

Fig. 6. The effect of pH on the reaction rate. The buffer systems and assay condition were the same as described under Fig. 5. To obtain the points, the pH was determined at the end of 2-min reaction.

TABLE II

EFFECTS OF ACTIVATORS AND INHIBITORS ON D-XYLOSE REDUCTASE

The assay method was used as described in the text, except that 0.1 ml of 0.15 *M* D-xylose and 5 units purified enzyme were used. The readings were taken from the initial velocity after the first minute.

Compound	*Final concentration (M)*	*Change in O.D. at 340 mμ*	*% Activity*
None		0.080	100
Glutathione	$1 \cdot 10^{-3}$	0.080	100
Cysteine	$1 \cdot 10^{-2}$	0.080	100
p-Chloromercuribenzoate	$5 \cdot 10^{-4}$	0.040	50
	$1 \cdot 10^{-4}$	0.060	80
p-Chloromercuribenzoate + glutathione	$5 \cdot 10^{-4}$ $5 \cdot 10^{-4}$	0.080	100
p-Chloromercuribenzoate + cysteine	$5 \cdot 10^{-4}$ $5 \cdot 10^{-4}$	0.068	85
Potassium floride	$1 \cdot 10^{-2}$	0.071	90
	$5 \cdot 10^{-2}$	0.043	54
Cupric sulfate	$1 \cdot 10^{-2}$	0.071	90
Zinc sulfate	$1 \cdot 10^{-3}$	0.011	14
	$7 \cdot 10^{-4}$	0.033	41
	$5 \cdot 10^{-4}$	0.067	84
	$1 \cdot 10^{-4}$	0.080	100

References p. 463.

TABLE III

COMPARISON OF THE RATES OF REDUCTION OF DL-GLYCERALDEHYDE AND D-XYLOSE BY ENZYME PREPARATIONS

Substrate	Crude preparation		Purified preparation	
	Specific activity	Ratio to xylose	Specific activity	Ratio to xylose
DL-Glyceraldehyde	270	7.5	2200	2.9
D-Xylose	36	1	750	1

TABLE IV

REDUCTION OF ALDOPENTOSES BY PURIFIED D-XYLOSE REDUCTASE

The condition was the same as the standard assay method except that 15 μmoles substrate and 5 units purified enzyme were used. The readings were taken from the initial velocity after the first min.

Substrate	Change in O.D. at 340 mμ	% Activity
D-Xylose	0.08	100
L-Xylose	0	0
D-Arabinose	0	0
L-arabinose	0.02	25
D-Ribose	0.02	25

at $5 \cdot 10^{-2}$ M. There was no effect by $1 \cdot 10^{-2}$ M versene or $1 \cdot 10^{-3}$ M dipicolinic acid. Among the metals tested at a final concentration of $1 \cdot 10^{-3}$ M, Zn^{++} was a strong inhibitor, while Cu^{++} showed a 10% inhibition and Mg^{++}, Ca^{++}, and Mn^{++} had no effect.

Substrate specificity: This 20-fold purified enzyme preparation contained a considerable amount of enzyme which oxidized TPNH in the presence of DL-glyceraldehyde. The change in ratio of enzymic activities during purification showed that the enzyme which reduced DL-glyceraldehyde was distinct from D-xylose reductase (Table III). Among the aldopentoses tested, in addition to D-xylose,

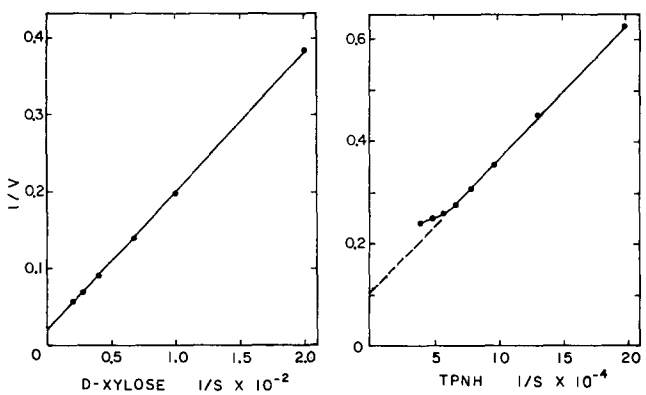

Fig. 7. The LINEWEAVER-BURK plot of D-xylose and TPNH. The assay condition was the same as described and 5 units of enzyme protein were used.

References p. 463.

L-arabinose and D-ribose were found to serve as substrates but at a rate only one-fourth that on D-xylose (Table IV). TPNH was the only active coenzyme. The k_s calculated from a LINEWEAVER AND BURK[6] plot was 0.09 M for D-xylose and $2.2 \cdot 10^{-5}$ for TPNH (Fig. 7, the tailing in the TPNH plot resulted from D-xylose being the limiting factor in the assay system). The enzyme preparation did not react withh D-glucose, D-galactose, D-mannose, D-rhamnose, D-glucuronic acid or D-galacturonic acid.

Reversibility: At pH 7.0, the equilibrium of D-xylose reductase favors the complete reduction of D-xylose. There was a detectable rate of the reverse reaction when the enzyme was incubated with TPN and a concentration of D-xylitol 1 M. However, at pH 9.0 and with a high concentration of D-xylitol, the reverse oxidation proceeded in a slightly faster rate.

DISCUSSION

It appears that the initial step in D-xylose metabolism by cell-free extracts of *P. chrysogenum* is the conversion of D-xylose to D-xylulose, resulting from two enzymic reactions with the formation of D-xylitol as an intermediate. It is possible that this pathway of interconversion is operating exclusively in the intact cell, since all attempts to find indications of other reactions in cell-free extracts were unsuccessful.

Accordingly, the reactions proceed as follows:

$$\text{D-xylose} + \text{TPNH} + \text{H}^+ \longrightarrow \text{D-xylitol} + \text{TPN}^+$$
$$\text{D-xylitol} + \text{DPN}^+ \rightleftharpoons \text{D-xylulose} + \text{DPNH} + \text{H}^+$$
$$\overline{\text{D-xylose} + \text{TPNH} + \text{DPN}^+ \rightleftharpoons \text{D-xylulose} + \text{TPN}^+ + \text{DPNH}}$$

It is interesting to note that the overall conversion involves a transfer of hydrogen from TPNH to DPN. The generation of DPNH resulting from reoxidation of TPNH by mediation of a substrate provides more energy for the cell, since the oxidation of TPNH by way of cytochrome system, in contrast to that of DPNH, is less coupled to phosphorylation[7,8]. An analogous observation has been reported in mammalian tissues where the synthesis of fructose by certain male accessory sexual tissues involves the reduction of glucose to sorbitol by TPNH, followed by DPN-linked oxidation of sorbitol to fructose[9,10], in which there is a stoichiometric transfer of hydrogen from TPNH to DPN.

The TPNH-linked enzyme, for which the name D-xylose reductase is proposed, appears to be inducible, since extracts of cells grown on a D-glucose-synthetic medium are devoid of activity. The enzyme is relatively specific for D-xylose, although some activity was observed when D-ribose and L-arabinose were substrates. When cells of *P. chrysogenum* were grown on a synthetic medium with either D-xylose or D-glucose as the sole carbon source, cell-free extracts contained considerable activity for the enzymic oxidation of TPNH in the presence of DL-glyceraldehyde or D-erythrose. These enzymes are different from the D-xylose reductase described here since the latter is induced only in the presence of D-xylose. Somewhat analogous TPNH-linked polyol dehydrogenase systems have been reported in the extract of silkworm haemolymph and it was suggested that they might contribute the maintenance of the redox potential of the tissue[11]. Determining the significance of this group of enzymes in *P. chrysogenum* will require further studies.

References p. 463.

The variation in activity of the DPN-linked enzyme for xylitol oxidation in different batches of crude extract from *P. chrysogenum* was presumed to be caused by variations in the degree of cell and particle breakage. HOLLMANN AND TOUSTER[12] have obtained soluble L-xylulose-xylitol and other polyol dehydrogenases from the insoluble portion of ruptured guinea pig-liver mitochondria by butanol extraction. It is likely that in the cell-free extracts of *P. chrysogenum* the enzyme is associated to some particulate component. Our results also indicate that the equilibrium catalyzed by this enzyme favours the reduction of xylulose; this property differs from similar enzyme systems found in extracts of *Acetobacter suboxydans*, *Candida utilis*, and *Pseudomonas* species, which oxidize polyols readily[13,14].

SIH *et al.*[15] have demonstrated the pentose cycle reactions in cell-free preparations of *P. chrysogenum*; therefore, it is expected that the D-xylulose, formed from the conversions presented in this paper, might enter into the pentose cycle for subsequent metabolism after phosphorylation by ATP to D-xylulose-5-phosphate. Since this mold had very strong adenosine triphosphatase activity, there are no conclusive results on this phosphorylation at the present time; however, the amount of D-xylulose formed from the oxidation of D-xylitol by incubation of the crude extract and DPN was increased considerably by adding ATP.

ACKNOWLEDGEMENTS

We offer grateful thanks to Dr. W. A. WOOD for his valuable suggestions and samples of xylulose and to Dr. C. J. SIH for his discussions and assistance. This work was supported by grant G-3359 from the National Science Foundation.

REFERENCES

1 C. CHIANG, C. J. SIH AND S. G. KNIGHT, *Biochim. Biophys. Acta*, 29 (1958) 664.
2 Z. DISCHE AND E. BORENFREUND, *J. Biol. Chem.*, 192 (1951) 583.
3 B. L. HORECKER, P. A. SMYRNIOTIS AND H. KLENOW, *J. Biol. Chem.*, 205 (1953) 661.
4 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
5 L. P. ZILL, J. X. KHYN AND G. M. CHANIAE, *J. Am. Chem. Soc.*, 75 (1953) 1339.
6 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56, (1934) 658.
7 N. O. KAPLAN M. N. SCHWARTZ M. E. FRECH AND M. M. CIOTTI *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 481.
8 P. V. VIGNAIS AND P. M. VIGNAIS, *J. Biol. Chem.*, 229 (1957) 265.
9 H. G. HERS, *Biochim. Biophys. Acta*, 22 (1956) 203.
10 H. G. WILLIAMS-ASHMAN, J. BANKS AND S. K. WOLFSON, JR., *Arch. Biochem. Biophys.*, 72 (1957) 485.
11 P. FAULKNER, *Biochem. J.*, 68 (1958) 374.
12 S. HOLLMANN AND O. TOUSTER, *J. Biol. Chem.*, 225 (1957) 87.
13 A. C. ARCUS AND N. L. EDSON, *Biochem. J.*, 64 (1956) 385.
14 D. R. D. SHAW, *Biochem. J.*, 64 (1956) 394.
15 C. J. SIH, P. B. HAMILTON AND S. G. KNIGHT, *J. Bacteriol.*, 73 (1957) 447