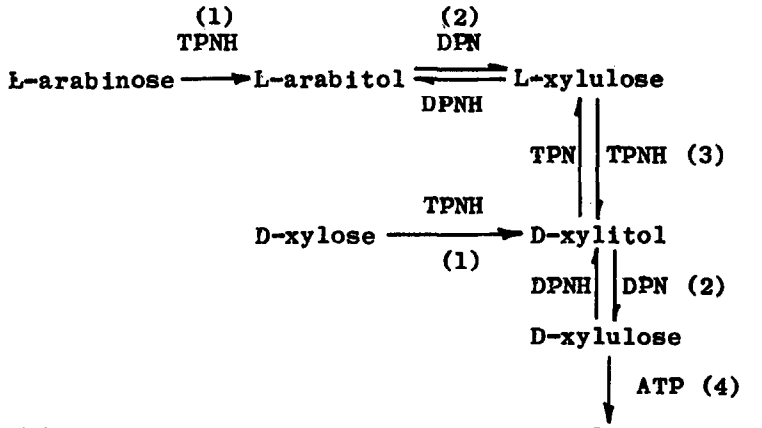
A NEW PATHWAY OF PENTOSE METABOLISM*

Ching Chiang and S. G. Knight

Department of Bacteriology, University of Wisconsin
Madison, Wisconsin

Received November 9, 1960

From studies with cell-free extracts of Penicillium chrysogenum cells grown on the appropriate pentose it has been shown that the aldopentose is reduced to pentitol by a TPNH-linked reductase, followed by the oxidation of the pentitol to either D-xylulose or L-xylulose by a DPN-linked dehydrogenase (Chiang, Sih, and Knight, 1958; Chiang and Knight, 1959, 1960a). As a result of further studies the following new pathway of pentose metabolism is proposed and the enzyme systems are tentatively named:



(1) Aldopentose reductase
(2) Pentitol-DPN dehydrogenase
(3) L-xylulose-TPNH reductase
(4) D-xylulokinase

* This investigation was supported by Grant E-1201 from the National Institutes of Health.

The D-xylulokinase activity could be followed by measuring the disappearance of D-xylulose in extracts containing ATP. During the course of incubation, D-xylulose was phosphorylated and was precipitated in the form of the barium salt by the Somogyi Zn-Ba precipitating reagent (Somogyi, 1945); the residual D-xylulose was analyzed by the cysteine-carbazole method (Dische and Borenfreund, 1951). Table 1 shows that approximately the same xylulokinase activity occurred in extracts of cells grown on either D-xylose or L-arabinose. Several phosphorylated sugars were detected in the reaction mixture before the Zn-Ba precipitation. D-Xylulose-5-phosphate was found as the major product

Table 1

The disappearance of D-xylulose when incubated with ATP in extracts from cells grown on D-xylose or L-arabinose

Time in minutes	Percentage utilized by extracts of cells grown on	
	D-xylose	L-arabinose
10	26.6	21.4
20	32.1	27.6
40	43.4	33.7
60	48.8	43.1

The reaction mixture contained 5 µmoles D-xylulose, 15 µmoles ATP, 10 µmoles $MgCl_2$, 15 µmoles glutathione, 74 µmoles $KF \cdot 2H_2O$, 300 µmoles Tris buffer, pH 7.5, and 10 mg extract protein in a total volume of 0.8 ml. The temperature was 30 C.

by analysis on a Dowex-1 formate column; as incubation continued this sugar was converted to other phosphorylated sugars in amounts detectable by the transketolase-transaldolase reactions. The details of the studies on the phosphorylated sugars will appear

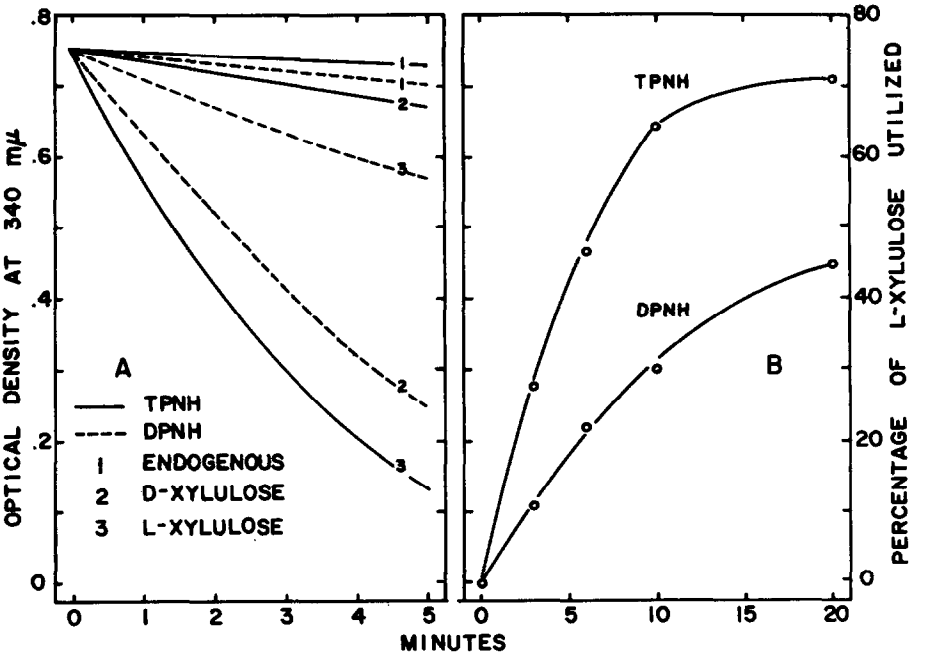


Fig. 1. Reaction of TPNH and DPNH with xyluloses. In A, the cuvette contained 10 μmoles D- or L-xylulose, 0.12 μmoles TPNH or DPNH, 50 μmoles Tris buffer, pH 7.4, and 90 μg extract protein in a total volume of 1 ml. In B, the mixture contained 5 μmoles L-xylulose, 10 μmoles TPNH or DPNH, 10 μmoles $MgCl_2$, 300 μmoles Tris buffer, pH 7.5 and 10 mg extract protein in a total volume of 0.9 ml. The temperature was 30°C.

in a later report. Under the same experimental conditions neither extract catalyzed the phosphorylation of L-xylulose, L-ribulose, D-xylose, L-arabinose, xylitol or L-arabitol.

Since D-xylulose is the only substrate for phosphorylation by the kinase and ATP, the L-xylulose formed as the major oxidation product of L-arabitol by the DPN-linked dehydrogenase must be converted in some way to D-xylulose. A TPNH-linked, L-xylulose reductase has now been found in the extracts of cells grown on L-arabinose. Several lines of evidence suggest that this TPNH-linked enzyme differs from the DPN-linked pentitol dehydrogenase. (1) The extracts catalyze the oxidation of TPNH with L-xylulose about 18 times faster than with D-xylulose, while DPNH oxidation is the opposite and the reaction rate with

D-xylulose was about 4 times greater than with L-xylulose (Fig. 1A). (2) Incubation of the extracts with L-xylulose and either DPNH or TPNH (in a ratio of 1:2) gave different rates of L-xylulose utilization. This could indicate the presence of two enzymes in the extracts with different affinites for the sugar (Figure 1B). (3) The identification of the products from the reduction of L-xylulose indicated that xylitol was produced with TPNH and arabitol with DPNH. Identifications were made by placing the concentrated deionized reaction mixture, which had been inactivated by heating in a boiling water bath and treated with charcoal, on Whatman No. 1 filter paper and developing with 80% aqueous isopropanol at 30°C for 24 hours. The sugars were located by alkaline silver nitrate. Spots corresponding to xylitol and arabitol were obtained from the reaction mixture but not in the control without the addition of the reduced coenzymes. (4) There were differences in reaction rates for TPN or DPN reduction at an alkaline pH by the extracts. The TPNH-linked L-xylulose reductase system gave an optical density change of 0.089 at 340 mμ per minute on 0.1 M D-xylitol, 0.008 on 0.1 M L-arabitol, and 0.001 on 0.1 M D-adonitol; indicating a high specificity for D-xylitol; this was consistent with previous observations on TPNH oxidation with L-xylulose. It also is unlikely that the TPN reduction by D-xylitol is the reverse reaction of the aldopentose reductase, since the enzyme is not reactive with L-arabitol. In contrast, in the reduction of DPN, it is the pentitol-DPN dehydrogenase system which reacts on all three pentitols with rather similar reaction rates, although the rate on D-xylitol was about three times faster than with the others (Table 2). By incubating the enzyme with a mixture of substrates it appeared that a single enzyme catalyzes these reactions, since the reaction rate was not greater than

with one substrate. According to this experimental evidence, the TPNH-L-xylulose reductase and the pentitol-DPN dehydrogenase differ from each other. Similar enzyme systems have been found in the guinea pig liver mitochondria (Hollman and Touster, 1957).

Table 2

Reduction of DPN with pentitols

Addition	Optical density change/min.
L-Arabitol	0.050
D-Adonitol	0.042
Xylitol	0.150
L-Arabitol + D-Adonitol	0.056
L-Arabitol + Xylitol	0.158

The 1 ml reaction mixture contained 100 µmoles of each pentitol; however, only L-arabitol gave the maximum velocity at this concentration. 0.5 µmoles DPN, 100 µmoles Tris buffer, pH 8.5, and 0.3 mg extract protein were included.

One may ask whether the TPNH-linked L-xylulose reductase is the same as the TPNH-linked aldopentose reductase. Preliminary observations on the reactions with a mixture of substrates suggest that they are different enzyme systems. As shown in Table 3, the reaction rates with mixtures containing L-xylulose and either L-arabinose or D-xylose showed an additive effect, resulting from the sum of each reaction; this was not so with the mixture of aldopentoses.

The separation and purification of the above enzyme systems is being undertaken to obtain a better understanding of their properties.

Table 3

Oxidation of TPNH with pentoses

Addition	Optical density change/min.
L-Arabinose	0.100
D-Xylose	0.065
L-Xylulose	0.120
L-Arabinose + L-Xylulose	0.220
D-Xylose + L-Xylulose	0.185
D-Xylose + L-Arabinose	0.110

The reaction conditions were as in Fig. 1A except that 0.1 M of each aldopentose and 0.01 M L-xylulose was the final concentration.

The enzymic reduction of D-xylose and the oxidation of xylitol has been found in several other species of molds and yeasts (Chiang and Knight, 1960). Recently, Veiga *et al.* have also described similar enzyme systems in *Candida albicans*. These findings emphasize the significance of this new pathway for pentose metabolism in the filamentous fungi and yeasts.

References

Chiang, C., Sih, C. J., and Knight, S. G., Biochim. Biophys. Acta, 29, 664 (1958)

Chiang, C., and Knight, S. G., ibid, 35, 454 (1959)

Chiang, C., and Knight, S. G., Bact. Proceedings, 1960a, 182; and Biochim. Biophys. Acta, in press.

Chiang, C., and Knight, S. G., Nature, 188, 79 (1960)

Dische, Z., and Borenfreund, E., ibid, 192, 583 (1951)

Hollman, S., and Touster, O., ibid, 225, 87 (1957)

Somogyi, M., J. Biochem. 160, 69 (1945)

Veiga, L. A., Bacila, M., and Horecker, B. L., Biochem. Biophys. Res. Comm. 2, 440 (1960)