# PENTOSE METABOLISM IN CANDIDA ALBICANS. I. THE REDUCTION OF D-XYLOSE AND L-ARABINOSE*

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Resting cells of Candida albicans, grown on xylose, utilize this sugar by oxidative processes, but are unable to ferment it (Veiga, 1959). It has now been shown that extracts of such cells contain an active TPN-linked dehydrogenase, which catalyzes the reaction:

$$\underline{D}\text{-Xylose} + \text{TPNH} + H^+ \rightleftharpoons \text{Xylitol} + \text{TPN}^+$$

The reaction is most readily measured by following the oxidation of TPNH with xylose (Fig 1A), but at alkaline pH and in the presence of high concentrations of xylitol, it can be observed to proceed in the direction of xylitol oxidation (Fig 1B).

Extracts of xylose-grown cells also catalyze the oxidation of TPNH by L-arabinose and the reduction of TPN by L-arabitol (Fig 1A and 1B). Oxidation of TPNH is also observed with D-ribose, but not with D-arabinose, or D or L-lyxose. TPN is not reduced with D-arabitol. Several lines of evidence suggest that the reactions with D-xylose, L-arabinose and D-ribose are catalyzed by a single enzyme: (1) The

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ratio of activities remains unchanged during fractionation with

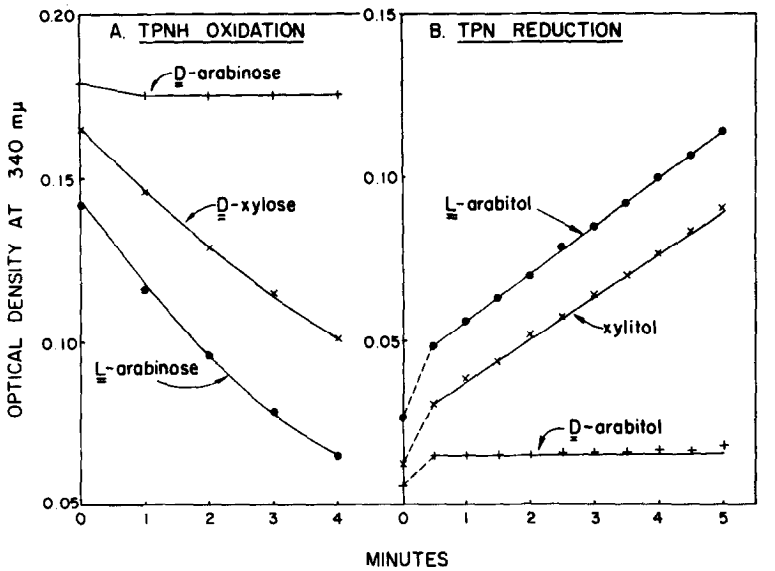


Figure 1. Reaction of TPN with pentoses and pentitols. The reaction cuvettes in A contained 10 μmoles of pentose, 0.1 μmole of TPNH and 0.1 M glycylglycine buffer, pH 7.3 in a total volume of 3.0 ml. In B the cuvettes contained 10 μmoles of pentitol, 0.1 μmole of TPNH and 0.03 M glycine buffer, pH 8.6, in a total volume of 3.0 ml. The reactions were begun by the addition of 0.1 mg of an ammonium sulfate fraction (see Table I) in A and 0.25 mg in B. The temperature was 23°.

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ammonium sulfate or following precipitation with acid (Table I). (2) The reaction rate with two substrates added together is no greater than with either substrate alone (Table II). (3) Extracts of glucose-grown cells show no activity with either D-xylose or L-arabinose; both activities appear when cells are grown on either D-xylose or L-arabinose. Further purification of the enzyme will be undertaken, in order to determine whether a single enzyme catalyzes the reduction of all three pentoses.

In the case of xylitol oxidation the product has been isolated and identified as D-xylose. This was accomplished with catalytic quantities of TPN by coupling the reaction to the reduction of pyruvate in the

Table I

Fractionation of Candida Extracts

| Fraction* | Specific activity** with | | |
|---|---|---|---|
| | D-xylose | L-arabinose | D-ribose |
| Extract | 0.044 | 0.043 | 0.023 |
| Ammonium sulfate | 0.109 | 0.106 | 0.041 |
| pH precipitate | 0.185 | 0.254 | 0.069 |

* The extract was prepared by autolysis of air-dried yeast for 16 hours at 30° in 3 parts (w/v) of 0.1 M $NaHCO_3$. The ammonium sulfate fraction was collected between 60 and 70 °/o saturation. The final fraction was the precipitate formed when the pH was lowered from 6.5 to 4.0 in the presence of ammonium sulfate at 50 °/o saturation.

** The test system is that described in Fig 1A. Specific activity is the oxidation of TPNH in density units per minute per mg of enzyme.

Table II

Reaction with Mixtures of Substrates

| Addition† | Density change per minute |
|---|---|
| D-Xylose | 0.0086 |
| L-Arabinose | 0.0098 |
| D-Ribose | 0.0060 |
| D-Xylose + L-arabinose | 0.0100 |
| D-Xylose + D-ribose | 0.0084 |

† The reaction conditions were as in Fig 1A except that the concentration of each sugar was 0.1 M, sufficient to saturate the enzyme. $K_s$ for D-xylose is approximately 0.02 M. Each cuvette contained 8 micrograms of ammonium sulfate fraction.

presence of lactic dehydrogenase. In a reaction mixture containing 10 μmoles of xylitol, 18 μmoles of pyruvate, 0.2 μmoles of TPN, 20 mg of rabbit muscle lactic dehydrogenase and 0.5 mg of the Candida enzyme (pH precipitate, see Table I), 4.1 μmoles of pentose were formed in 2 hours, as measured with the orcinol reaction (Mejbaum, 1939). Xylose was identified by paper chromatography in phenol-$H_2O$ and by its reaction with a specific xylose isomerase purified from extracts of Lactobacillus plantarum (Burma and Horecker, 1958). This enzyme will convert D-xylose quantitatively to D-xylulose in the presence of borate buffer and is inert with D-arabinose and other pentoses. Assay of the reaction mixture by the cysteine carbazole reaction (Dische and Borenfreund, 1951) showed it to contain 3.82 μmoles of D-xylose, in agreement with the quantity of pentose estimated to be present by the orcinol test.

The function of this enzyme in D-xylose and L-arabinose metabolism remains to be established. Extracts of xylose-grown cells also contain an enzyme which catalyzes the reduction of DPN by xylitol, however DPNH is oxidized by D-xylulose but not by D-xylose, suggesting that the product of this oxidation is different from that formed in the reaction with TPN. Therefore the initial steps in xylose metabolism may be as follows:

$$\text{D-xylose} \xrightarrow{\text{TPNH}} \text{D-xylitol} \xrightarrow{\text{DPN}} \text{D-xylulose}$$

The extracts are devoid of xylose isomerase. This represents, therefore, a novel pathway for the metabolism of aldoses, in which the ketose is formed by way of the sugar alcohol rather than by direct isomerization. A similar pathway for xylose metabolism has been described in extracts of Penicillium chrysogenum (Chiang, Sih, and Knight, 1958). Extracts of this organism are also active with both D-xylose and L-arabinose (Chiang, C.

and Knight, S. G. 1960).

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