MODE OF FORMATION OF D-ARABITOL BY SACCHAROMYCES MELLIS

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Osmophilic yeasts produce D-arabitol and glycerol when growing aerobically on glucose in a medium in which the amount of phosphate is low and the ratio of carbohydrate to nitrogenous compounds is high (Spencer and Shu, 1957; Peterson et al., 1958; Hajny et al., 1960; Ōnishi and Saito, 1961). Spencer et al. (1956) determined the labeling pattern in D-arabitol produced by a fast-growing osmophile from glucose labeled in position 1 or 2. They concluded that the sugar alcohol probably originated from compounds in the pentose-phosphate cycle. The present work was undertaken as an attempt to provide a possible enzymatic explanation for pentitol production by osmophilic yeasts. Saccharomyces mellis NRRL Y-1053 was selected as the organism with which to study this reaction since it has been shown (Peterson et al., 1958) that the major portion of the polyalcohols produced by this organism was D-arabitol.

Extracts of S. mellis, obtained by sonic irradiation, contain a TPN-linked enzyme that oxidizes D-arabitol to D-ribulose. The pH optimum for oxidation is around 10 (Fig. 1). The rate of the reverse reaction, reduction of D-ribulose with TPNH, increased as the pH was lowered (Fig. 2). The rate below pH 5.5 could not be measured since the crude enzyme extract became turbid under these more acidic conditions.

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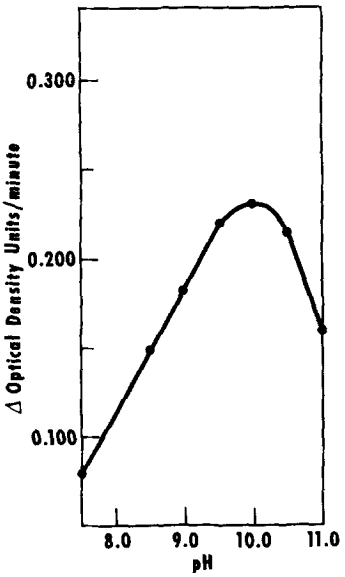

Fig. 1. Optimum pH for D-arabitol oxidation. Each cuvette contained 0.8 ml 0.1 M NH_4OH-NH_4Cl buffer of the pH indicated, 0.1 ml 0.01 M TPN, 0.1 ml 0.3 M D-arabitol, and 0.1 ml crude extract.

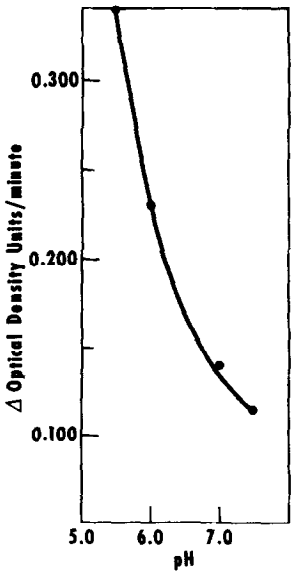

Fig. 2. Rate of D-ribulose reduction as a function of pH. Cuvettes contained 0.9 ml 0.1 M phosphate buffer of the pH indicated, 0.05 ml 0.004 M TPNH, 0.05 ml 0.05 M D-ribulose, and 0.05 ml crude extract. (D-Ribulose was a gift of Dr. G. Ashwell.)

D-Arabitol dehydrogenase showed no metal requirements. It was not inhibited by EDTA[1] nor did dialysis have any effect on the reaction. The oxidation was studied in NH_4OH-NH_4Cl buffer. Tris buffer depressed the rate of the reaction, and borate was completely inhibitory at concentrations of 0.1 M. On the other hand, the rate of TPN reduction in pyrophosphate and glycine buffers was the same as in NH_4OH buffer. Reagents usually employed to react with sulfhydryl groups (glutathione, Na_2S, BAL, PCMB, IOA and arsenite) neither inhibited nor stimulated the reaction.

Other polyols and sugars were suitable substrates for crude cell extracts under the same conditions. However, D-arabitol oxidation and D-ribulose reduction appear to be catalyzed by a separate enzyme since this was the only system specific for TPN and TPNH as the hydrogen carrier. Xylitol oxidation was catalyzed by an enzyme system that could use both DPN and TPN though the rate of the reaction was four times greater with DPN. Reduction of D-xylulose showed the same relative preference for DPNH over TPNH. L-Xylulose and fructose reductions proceeded quite slowly and were specific for DPNH. Sorbitol, ribitol, and dulcitol were also oxidized by these extracts by a DPN-requiring system. L-Arabitol, mannitol, glycerol, L-sorbose, and all aldopentoses and aldohexoses tested were inactive as substrates.

The keto sugar produced by the oxidation of D-arabitol was prepared in larger quantities for identification purposes by coupling the reaction with the glutamic dehydrogenase also in S. mellis extracts. In a medium composed of 1.0 ml 0.3 M D-arabitol, 1.0 ml 1.0 M ammonium α-ketoglutarate, 0.5 ml 1.0 M NH_4OH, 0.2 ml 0.01 M TPN, 0.5 ml crude extract, and 1.8 ml 1.0 M Tris, pH 8.5, the reaction was complete in about 8 hours with 50% of the arabitol oxidized, as determined by the cysteine-carbazole reaction (Dische and Borenfreund, 1951). The reaction mixture was treated with IR-120(H^+) to remove cations and then chromatographed in butanol-1:pyridine:water (6:4:3). The

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[1] Abbreviations used: G1-6-P, glucose-6-phosphate; 6-P-G1, 6-phosphogluconate; D-Ru-5-P, D-ribulose-5-phosphate; D-Ru, D-ribulose; Tris, tris(hydroxmethyl) aminomethane; EDTA, ethylenediamine-tetraacetate; BAL, 2,3-dimercapto-1-propanol; PCMB, p-chloromercurebenzoate; IOA, iodoacetate.

reactants were detected by spraying the sheet with either silver nitrate (Trevelyan et al., 1950) or urea phosphate (Greene, 1958). The keto sugar co-chromatographed with ribulose and separated from xylulose. Treatment of the reaction mixture with D-arabinose isomerase from Aerobacter aerogenes resulted in a disappearance of keto sugar while the isomerases for L-arabinose and D-xylose had no effect. Thus it can be concluded that the keto sugar produced from D-arabitol is D-ribulose.

A possible pathway for D-arabitol formation might be as follows:

$$\text{Glucose} \xrightarrow{ATP} \text{Gl-6-P} \xrightarrow{TPN} \text{6-P-Gl} \xrightarrow{TPN} \text{D-Ru-5-P} \longrightarrow \text{D-Ru} \overset{P}{} \xrightleftharpoons{TPNH} \text{D-Arabitol}$$

The requisite enzymes have all been detected in S. mellis extracts. However, this is probably not the only mechanism for arabitol formation. The results by Spencer et al. (1956) suggest that a so-called reversal of the pentose-phosphate cycle may play a significant role in arabitol formation. One possible function of arabitol formation would be to regenerate the TPN reduced by the oxidation of Gl-6-P to D-Ru-5-P. However, if the scheme presented here was the only one for ribulose formation, only half of the reduced TPN could be regenerated. Therefore, it is necessary to invoke a second pathway for D-Ru-5-P formation so that the remaining TPNH can be reoxidized. Synthesis of this keto-pentose via transaldolase-transketolase-catalyzed reactions would satisfy this requirement.

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