# A GUANOSINE DIPHOSPHATE ALDOHEPTOSE IN YEAST

Victor Ginsburg

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

Received August 8, 1960

GDP-D-mannose was isolated from baker's yeast (Cabib and Leloir, 1954) for studies on the biosynthesis of GDP-L-fucose by Aerobacter aerogenes. Analysis of the GDP-D-mannose did not reveal any aldohexose component other than mannose or any 6-deoxyhexose (Ginsburg, 1960). Analysis of a second preparation revealed a trace component amounting to approximately 2% of the total reducing sugar, calculated as mannose, liberated from the nucleotide by mild acid hydrolysis. The unknown sugar, which migrated slightly slower than glucose, could be separated completely from mannose by paper chromatography using ethyl acetate-pyridine-water, 3.6:1.0:1.15, as a solvent. In a commercial sample of GDP-D-mannose prepared from baker's yeast (Sigma Chemical Co., St. Louis, Mo.), the same component was observed in higher quantity (8% of the total reducing sugar liberated by hydrolysis). From 16 μmoles of this GDP-D-mannose, 0.7 μmole of the unknown sugar (calculated as mannose) was isolated chromatographically. The isolated sugar exhibited a characteristic 510 mμ absorption peak in a test for heptoses (Dische, 1953) which was identical in shape to those given by authentic heptoses. That the unknown sugar is an aldose and not a ketose was demonstrated by the fact that treatment with bromine water completely destroyed its ability to produce this peak (Slein and Schnell, 1953). Further evidence that the unknown sugar is an aldoheptose was indicated by the characteristic color it exhibited on paper when sprayed with p-anisidine hydrochloride (Davies, 1957A).

Of the 32 possible aldoheptoses, 3 have been identified as components of bacterial polysaccharides; L-glycero-D-mannoheptose in Shigella flexneri and Escherichia coli (Slein and Schnell, 1953; Weidel, 1955), D-glycero-D-galacto-heptose in Chromobacterium violaceum (Bn) (MacLennan and Davies, 1957), and what is probably D-glycero-D-mannoheptose in Chr. violaceum (NCTC 7917) (Davies, 1957B).

The chromatographic properties of the sugar from yeast were compared with authentic samples of aldoheptoses which were kindly supplied by Dr. N. K. Richtmeyer. Even though all the possible aldoheptoses are not available for comparison, the unknown sugar could be tentatively identified as D-glycero-D-mannoheptose (or its optical enantiomorph) by using the solvent systems and data of Davies (1957A). Additional evidence for the identity of this sugar is supplied by the fact that treatment with 0.05 M phosphate buffer, pH 7.4, for 1 hour at 100° caused its partial conversion to two new sugars as revealed by paper chromatography using 2-butanone-acetic acid-saturated boric acid solution, 9:1:1. Under the same conditions, authentic D-glycero-D-mannoheptose also yielded two new sugars with the same chromatographic mobilities as those obtained from the unknown sugar. Relative to the original aldoheptose, the mobilities of the new sugars were 0.6 and 1.3 which are the mobilities in this solvent system of D-glycero-D-glucoheptose and sedoheptulose, respectively. The latter two sugars would be expected to arise from the epimerization of D-glycero-D-mannoheptose. With 95% acetone as a solvent, the epimerization products from both the unknown sugar and D-glycero-D-mannoheptose had mobilities relative to the original aldoheptose of 0.7 and 1.9. Again, these values correspond to D-glycero-D-glucoheptose and sedoheptulose, respectively.

Although the heptose-containing nucleotide could not be separated from GDP-D-mannose by chromatography, it could be partially purified by using an enzyme preparation from A. aerogenes. The heptose nucleotide is not metabolized by extracts of A. aerogenes which convert GDP-D-mannose to intermediates in the pathway of GDP-L-fucose biosynthesis. These intermediates,

which appear to be GDP-4-keto-6-deoxyhexoses, can be converted by chemical reduction to GDP-6-deoxyhexoses (Ginsburg, in preparation). The heptose-containing nucleotide and residual GDP-D-mannose can then be readily separated from the GDP-6-deoxyhexoses by paper chromatography (Ginsburg, 1960). By this procedure, starting with 9 μmoles of commercial GDP-D-mannose, 0.3 μmole of a guanosine nucleotide was isolated which had the chromatographic property of GDP-D-mannose but which, on analysis, was found to contain 80% heptose and 20% mannose. The base moiety derived from this nucleotide by mild acid hydrolysis was chromatographically indistinguishable from GDP.

The results presented in this paper indicate that GDP-D-mannose isolated from yeast contains varying amounts of a new sugar nucleotide, a GDP-aldoheptose. The aldoheptose appears to be D-glycero-D-mannoheptose or its optical enantiomorph.

## References

Cabib, E., and Leloir, L. F., J. Biol. Chem., 206, 779 (1954).

Davies, D. A. L., Biochem. J., 67, 253 (1957A).

Davies, D. A. L., Nature, 180, 1129 (1957B).

Dische, Z., J. Biol. Chem., 204, 983 (1953).

Ginsburg, V., J. Biol. Chem., 235, 2196 (1960).

MacLennan, A. P., and Davies, D. A. L., Biochem. J., 66, 562 (1957).

Slein, M. W., and Schnell, G. W., Proc. Soc. Exptl. Biol. Med., 82, 734 (1953).

Weidel, W., Hoppe-Seyl. Z., 299, 253 (1955).