ENZYMATIC OXIDATION OF GUANOSINE DIPHOSPHOMANNOSE TO GUANOSINE DIPHOSPHOMANNURONATE

Jack Preiss

National Institutes of Health, Bethesda, Maryland

Received September 5, 1962

Knutson et al. (1) have described in a brief report the formation of an exocellular heteropolysaccharide composed of D-glucose, D-galactose, and mannuronic acid by the bacterium, Arthrobacter sp NRRL B-1973. Since crude-cell free extracts obtained by sonic oscillation of this microorganism contained a high titer of GDP-mannose pyrophosphorylase, it seemed that a possible mechanism for forming mannuronate would be by the direct oxidation of GDP-mannose to GDP-mannuronate. Indeed, incubation of GDP-mannose and DPN with the extract produced an increase of absorbance at 340 mμ. Upon fractionation of the crude extract with ammonium sulfate and then dialysis, it was found that the dehydrogenase activity resided in the 0.41-.90 fraction. At present, at least 60% of the added GDP-mannose can be oxidized (assuming 2 equivalents of DPN are reduced per mannuronate formed) with this dialyzed ammonium sulfate fraction. Omission of GDP-mannose resulted in no reduction of DPN; α-mannose-1-phosphate, UDP-glucose, and TDP-glucose could not substitute for GDP-mannose; TPN could not replace DPN.

In order to isolate the product formed, 21.8 μmoles of GDP-mannose were incubated with 5.0 millimoles of potassium phosphate pH 7.7, 110 μmoles of DPN, and 20 mg of the 0.41-0.90 ammonium sulfate fraction in 100 ml at room temperature. At the end of two hours, 18.5 μmoles of DPNH were formed. After 50 μmoles of sodium pyruvate and an excess of lactate dehydrogenase (Worthington) were added to reoxidize the formed DPNH, the reaction mixture contents were adsorbed on a Dowex 1-X8 chloride column. DPN and inorganic phosphate were eluted with 0.01 N HCl. The column was then subjected to a linear gradient com-

posed of 500 ml of 0.01 N HCl in the mixing chamber and 500 ml of 0.01 N HCl-0.25 N LiCl in the reservoir. Two peaks which appeared to be guanosine derivatives were eluted, pooled separately and then desalted by adsorption on Norit A and elution with a 50% ethanol-0.015 N NH_3 solution.

Peak I contained 10.4 μmoles of a guanosine derivative which co-chromatographed with GDP-mannose in both ethanol-M ammonium acetate (5:2) (Solvent I), and isobutyric-M NH_4OH (10:6) (Solvent II) solvent systems as well as by paper electrophoresis at pH 4.0. Peak II contained 9.0 μmoles of a guanosine derivative which chromatographed as one spot in Solvents 1 and 2 and on paper electrophoresis at pH 4.0. Its mobility on electrophoresis at pH 4.0 was faster than GDP-mannose and slower in Solvents 1 and 2.

The identification of Peak II as GDP-mannuronate is based on the following:

1. The ultraviolet absorption spectra of the material was identical in every detail to that of a guanosine derivate at pH 1, 7.0 and 12. Subjection of the compound to the carbazole and borate carbazole assays indicated the presence of a uronic acid. Chemical analysis of the material suggested that it contained 1 mole of GDP for every mole of uronic acid (Table I).

2. Treatment of the material with 0.01 N HCl for 15 minutes at 100° liberated the uronic acid which co-chromatographed with mannuronate in Solvent 1 and on paper electrophoresis at pH 4.0. The major part of the ultraviolet-containing material (78%) co-chromatographed with GDP in Solvents 1 and 2 and on paper electrophoresis at pH 4.0. Further evidence for a nucleoside diphosphate was obtained by the assay method of Kornberg and Pricer (Table I). Fifteen per cent of the ultraviolet-containing material co-chromatographed in the above solvent systems as GMP and the remainder as guanosine. The acid lability as well as the negligible reducing value of the nucleotide before hydrolysis indicated a glycosidic bond between the uronic acid and GDP.

The uronic acid isolated by paper chromatography in Solvent 1 was reactive in the naphthoresorcinol, carbazole, borate-carbazole and orcinol reactions showing the same relative reactivity in each assay as authentic mannuronic acid. The isolated uronic acid when lactonized co-chromatographed

TABLE I

Analysis of Peak II and its Acid Hydrolysis Products

Sample	Assay	Amount
		μmoles/ml
Intact nucleotide	Guanosine (ultraviolet assay)	1.15
	Acid labile phosphorus (2)	1.10
	Total phosphorus (2)	2.24
	Borate-carbazole as mannuronic acid (3)	1.07
	Reducing sugar (4) as mannuronic acid	0.0
	Reducing sugar after hydrolysis for 15 minutes at 100° in 0.01 N HCl	1.04
Isolated products of acid hydrolysis		
GDP	Guanosine	0.99
	Nucleoside diphosphate assay (5)	1.02
	Acid labile phosphorus	1.00
	Total phosphorus	1.90
	Orcinol as ribose (6)	0.96
Uronic acid	Carbazole as mannuronic acid (7)	0.88
	Borate-carbazole as mannuronic acid	0.95
	Orcinol as mannuronic acid (6)	0.95
	Naphthoresorcinol as mannuronic acid (8)	0.91

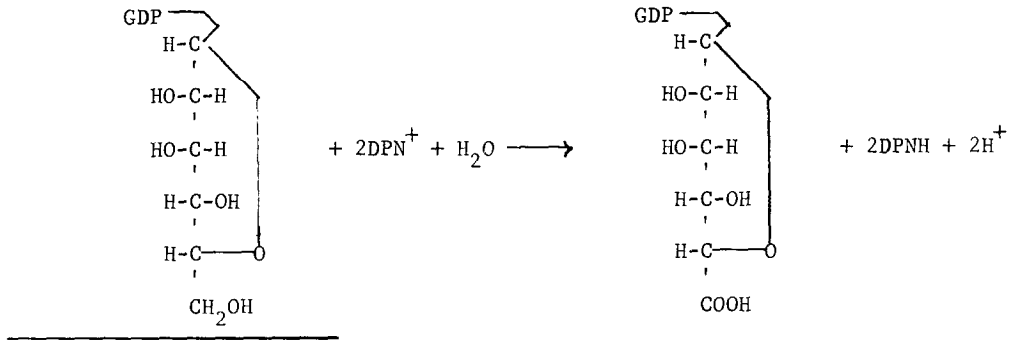
with mannuronolactone in a solvent system clearly separating it from the other naturally occurring uronic acid lactones, (ethyl acetate:pyridine:water, 40:11:6). Reduction of the uronic acid with sodium borohydride gave rise to a product whose lactone co-chromatographed in two solvent systems with mannonolactone (8). The borohydride reduction product when incubated with Escherichia coli extracts containing D-mannonate dehydrase (9) was converted

to ketodeoxygluconate as determined by the thiobarbiturate assay (10) and co-chromatography with authentic ketodeoxygluconate in two solvent systems (11)

Further evidence for the identification of the dehydrase product as ketodeoxygluconate was obtained by incubation of the product with ATP and E. coli extracts containing ketodeoxygluconate kinase and phosphoketodeoxygluconate aldolase (8). One mole of pyruvate was found per mole of product that disappeared. Since D-mannonate dehydrase is inactive with L-mannonate the configuration of the borohydride reduction product is D-mannonate and that of the isolated uronic acid D-mannuronic acid.

The stoichiometry of the formation of GDP-mannuronate is summarized in Figure 1. The suggestion is offered that GDP-mannuronate may be the glycosyl donor of mannuronic acid in the biosynthesis of the polysaccharide produced by the Arthrobacter.

Fig. 1. Enzymatic oxidation of GDP-mannose



D-Mannuronic acid is also present together with L-guluronic acid in the polymer, alginic acid, found in brown algae (12,13). It is quite possible that the same mechanism for synthesizing D-mannuronic acid occurs in this species.

This work was done in the laboratory of Dr. Gilbert Ashwell. His interest and his many helpful discussions of the problem are gratefully appreciated. I would also like to thank Doctors Allene Jeanes and Ralph F. Anderson of the

Northern Utilization Research and Development Division, United States Department of Agriculture, Peoria, Illinois, for providing the organism and directions for its growth before publication.

REFERENCES

1. Knutson, C. A., Watson, P. R., and Jeanes, A., The Division of Carbohydrate Chemistry of the American Chemical Society: Abstracts of Papers Presented at Washington, D. C., March 26-29, 1962, p. 4D.
2. Fiske, C. H., and SubbaRow, Y., J. Biol. Chem., 66, 375 (1925).
3. Gregory, J. D., Arch. Biochem. Biophys., 89, 157 (1960).
4. Nelson, N., J. Biol. Chem., 153, 375 (1944).
5. Kornberg, A., and Pricer, W. E., J. Biol. Chem., 182, 743 (1950).
6. Dische, Z., J. Biol. Chem., 204, 983 (1953).
7. Dische, Z., J. Biol. Chem., 167, 189 (1947).
8. Ashwell, G., Wahba, A. J., and Hickman, J., J. Biol. Chem., 235, 1559 (1960)
9. Smiley, J. D., and Ashwell, G., J. Biol. Chem., 235, 1571 (1960).
10. Weissbach, A., and Hurwitz, J., J. Biol. Chem., 234, 705 (1959).
11. Preiss, J., and Ashwell, G., J. Biol. Chem., 237, 317 (1962).
12. Fischer, F. G., and Dorfel, H., Hoppe-Seylers Z. fur Physiol. Chem., 302, 186 (1955).
13. Whistler, R. L., and Kirby, K. W., Hoppe-Seylers Z. fur Physiol. Chem., 314, 46 (1959).