The characterization of glycolipids derived from long-chain polyprenols: Chemical synthesis of α-D-mannopyranosyl dolichyl phosphate

ARTICLE *in* FEBS LETTERS · JUNE 1973

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(73)80134-6 · Source: PubMed

THE CHARACTERIZATION OF GLYCOLIPIDS DERIVED FROM LONG-CHAIN POLYPRENOLS: CHEMICAL SYNTHESIS OF α-D-MANNOPYRANOSYL DOLICHYL PHOSPHATE*

Christopher D. WARREN and Roger W. JEANLOZ**

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Mass. 02114, USA

Received 19 February 1973

1. Introduction

In bacteria many examples of the participation of long-chain polyprenols as lipid carriers of carbohydrate units in the biosynthesis of complex polysaccharides are known [1]; a "lipid intermediate" is formed, in which a carbohydrate residue is linked to a polyprenol residue through a mono- or pyrophosphate bridge. In the biosynthesis of yeast mannan, a similar mechanism appears to operate [2, 3] and the active lipid is probably dolichol; yeast dolichol is a mixture of very long-chain polyprenols, differing slightly in chain-length from mammalian dolichols [4], in which the main isomer is C_{95}. Recently, similar glycolipids, in which the lipid is either dolichol or a very similar compound, and where the carbohydrate being transferred is D-mannose [5–9], have been discovered as products of microsomal systems derived from a variety of animal tissues. The mannolipids have only been obtained in very small quantities, and this has made structural investigations difficult to perform. However, there are strong indications that the sugar is linked to the lipid through a monophosphate bridge. In an attempt to obtain definitive proof of the structure of the compound formed by pig-liver endoplasmic reticulum [10], we have performed a chemical synthesis of α-D-mannopyranosyl dolichyl phosphate by the DCC mediated condensation of dolichol with fully acetylated α-D-mannopyranosyl phosphate and subsequent deacylation of the product (scheme 1). The detailed comparison of the naturally formed and synthetic mannolipids is reported in the accompanying paper [10].

2. Materials and methods

Dolichol [2], isolated from pig liver, was a gift from Dr. F.W. Hemming. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl phosphate [1] was prepared from 1,2,3,4,6-penta-*O*-acetyl-α,β-D-mannopyranose, by a method [11] similar to that reported for the preparation of the D-galactose derivative [12]. The conditions for phosphodiester formation were similar to those used by Cawly and Letters [13]. Dicyclohexylcarbodiimide (DCC) was obtained from Eastman-Kodak, Rochester, N.Y. 14650.

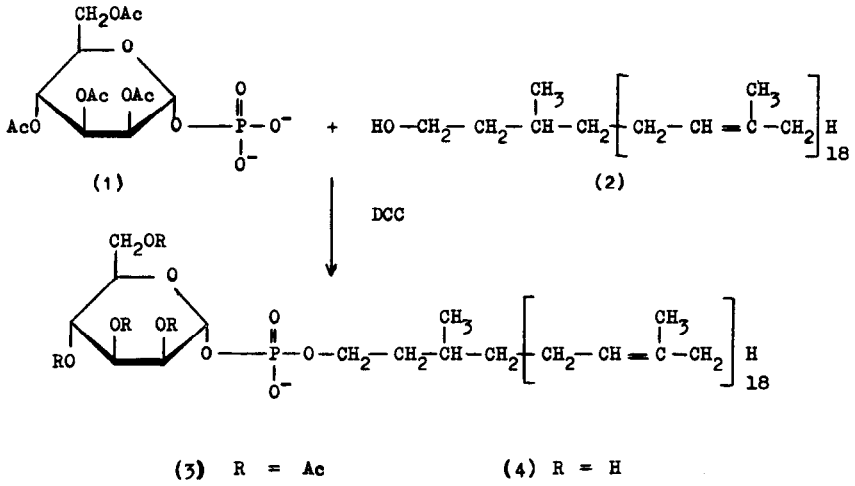
Optical rotations were determined in 1-dm semimicro tubes with a Perkin–Elmer Model 141 polarimeter. Infrared spectra were recorded with a Perkin–Elmer spectrophotometer, Model 237. Evaporations were carried out under reduced pressure, with an outside bath temp. kept below 30°.

Column chromatography was performed on Silica Gel 0.05–0.2 mm (70–325 mesh, E. Merck A.G., Darmstadt, Germany; the material was used without pretreatment). Thin-layer chromatography (TLC) was performed on precoated plates of Silica Gel G (Merck);

* This is publication No. 592 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This work was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases (AM 03564), National Institutes of Health, U.S. Public Health Service.

** To whom to address correspondence.

North-Holland Publishing Company – Amsterdam



Scheme 1.

the plates were cut to a length of 6 cm before use, otherwise they were used without pretreatment. Preparative TLC was carried out on precoated PLC plates, Silica Gel F 254 (Merck). The spots were detected with the anisaldehyde spray reagent [14] (a), the potassium permanganate spray reagent [15] (b), and the phosphate-specific spray reagent [16] (c). Solvents A, B and C were chloroform–methanol–water (60:25:4), (60:35:6) and (10:10:3), respectively, solvent D was 2,6-dimethyl-4-heptanone–acetic acid–water (20:15:2) and solvent E was 2-propanol–15 M ammonium hydroxide–water (6:3:1).

3. Results and discussion

A mixture of (2) (28 mg) and (1) (pyridinium form, 4 mg) was treated with DCC (20 mg). In order to remove traces of water, toluene (2 ml) was added to the mixture, and then evaporated, and this procedure was repeated three times. The anhydrous mixture was treated with dry pyridine (1 ml, kept for several days over calcium hydride) and stirred for 24 hr; additional amounts of DCC (20 mg) and (1) (4 mg) were added after 24 and 48 hr for a total reaction time of 72 hr. (The repeated addition and evaporation of toluene, followed by addition of dry pyridine as solvent was performed after each addition). The solvent was evaporated and the residue dissolved in chloroform (1 ml). TLC (a) indicated that more than 50% of the dolichol had been converted into (3). Column chromatography on silica gel (3.0 g) was performed with mixtures of chloroform and methanol, the proportion of methanol being gradually increased from (50:1), which eluted unchanged dolichol with some DCC and dicyclohexylurea, to (10:1) which eluted 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl dolichyl phosphate (3, pyridinium form, 21 mg). This material was deacylated in chloroform (0.5 ml) by treatment with 1% sodium methoxide in abs. methanol (0.5 ml). After 30 min TLC (solvent A, a) showed the formation of a new product, (R_f 0.54). Another substance, (R_f 0.8), with the same mobility as (3), was apparently a contaminant, as it was unaffected by a prolonged treatment with excess base. The reaction mixture was evaporated to ca. 0.2 ml and a few drops of chloroform added to give a clear solution. This was applied to a preparative TLC plate (20 cm × 5 cm) and eluted with solvent B. The band containing the compound (4) was located by spraying a narrow area near the center of the plate with (b) and (c), and the compound was extracted with solvent C. Evaporation of the solution gave syrupy α-D-mannopyranosyl dolichyl phosphate (4, 12 mg, sodium salt), $[\alpha]_D^{20}$ + 3.5° (*c* 0.8, chloroform–methanol, 5:1). It was pure on TLC in solvents A, B, D and E (a,b,c); the IR spectrum showed absorption maxima which were consistent with the expected structure: ν_{max}^{film} 3350

(very broad, OH), 2965 (CH_3 stretching), 2930 and 2860 (CH_2 stretching), 1730 (unassigned – also present in dolichyl phosphate [17]), 1660 (C=C stretching), 1450 (CH_2, $-CH_3$), 1376 ($-CH_3$), 1220 (P=O), 1070 (C–O– stretching, adjacent to a saturated isoprene residue), 975 and 835 cm^{-1} (–CH=C<); the NMR spectrum also showed most of the expected signals, and is given in the accompanying paper [10].

A small sample of the product (1 mg) was subjected to dilute acid hydrolysis with 0.5 ml of a mixture of M hydrochloric acid and methanol (1:10). Chloroform (approx. 0.2 ml) was added until a clear solution was obtained, which was then kept at 100° for 5 min in a sealed tube. Examination of the solution by TLC (a) showed the presence of only one substance derived from dolichol (an intense violet-blue spot) which co-chromatographed with dolichyl phosphate [17]: R_f 0.63 (solvent A) and 0.76 (solvent D). The other products were D-mannose (R_f 0.27) and methyl mannoside (R_f 0.43, R_f 0.55 and R_f 0.61) (solvent B), all of which gave a green spot.

Acknowledgement

We thank Dr. F.W. Hemming for the gift of dolichol.

References

[1] W.J. Lennarz and M.G. Scher, Biochim. Biophys. Acta 265 (1972) 417.
[2] W. Tanner, P. Jung and N.H. Behrens, FEBS Letters 16 (1971) 245.
[3] R. Sentandreu and J.O. Lampen, FEBS Letters 14 (1971) 109.
[4] P.H.W. Butterworth and F.W. Hemming, Arch. Biochem. Biophys. 128 (1968) 503.
[5] S.S. Alam, R.M. Barr, J.B. Richards and F.W. Hemming, Biochem. J. 121 (1971) 19P.
[6] J.B. Richards, P.J. Evans and F.W. Hemming, Biochem. J. 124 (1971) 957.
[7] R.M. Barr and F.W. Hemming, Biochem. J. 126 (1972) 1203.
[8] J.B. Richards and F.W. Hemming, Biochem. J. 30 (1972) 77.
[9] J.W. Baynes and E.C. Heath, Federation Proc. 31 (1972) 437.
[10] P.J. Evans and F.W. Hemming, FEBS Letters 31 (1973) 335.
[11] C.D. Warren and R.W. Jeanloz, in preparation.
[12] C.D. Warren and R.W. Jeanloz, Biochemistry 11 (1972) 2565.
[13] T.N. Cawley and R. Letters, Carbohyd. Res. 19 (1971) 373.
[14] P.J. Dunphy, J.D. Kerr, J.F. Pennock and K.J. Whittle, Chem. Ind. (London) (1966) 1549.
[15] J. Gigg and R.H. Gigg, J. Chem. Soc., C (1966) 82.
[16] J.C. Dittmer and R.L. Lester, J. Lipid Res. 5 (1964) 126.
[17] J.F. Wedgewood and C.D. Warren, Federation Proc., in press.