A PLANT MANNOSYL-LIPID ACTING IN REVERSIBLE TRANSFER OF MANNOSE

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1. Introduction

The biosynthesis of several bacterial cell wall [1–4] or membrane [5] polysaccharides occurs by transfer of sugar phosphate or sugar residues from nucleoside diphosphate sugars to a C_{55}-polyisoprenol phosphate followed by transfer of the carbohydrate unit to an acceptor to form the polymer. The glycolipid intermediates are formed in all cases from the nucleotide by reversible reactions [1–6]. This indicates that the sugar transfer potential of the nucleotide is preserved in the glycolipids which renders them in turn good donors for sugar transfer reactions occurring at membrane interphases. The experiments reported in this communication show that a glycolipid synthesized from GDP-mannose and an endogenous lipid by a particulate enzyme preparation derived [7] from mung bean shoots exhibits a similar group transfer potential. The lipophilic part of this compound is labelled from ^{3}H-mevalonic acid and, therefore, may be a polyprenol as in the bacterial systems [1–5].

2. Material and methods

The preparation of the particulate enzyme suspension from dark grown shoots of mung beans (*Phaseolus aureus*) has been described elsewhere [7]. All other materials and methods are included in the legends of the figures and the table.

3. Results and discussion

3.1. *The lipid part of the molecule*

The ^{14}C-mannose containing glycolipid was ex-

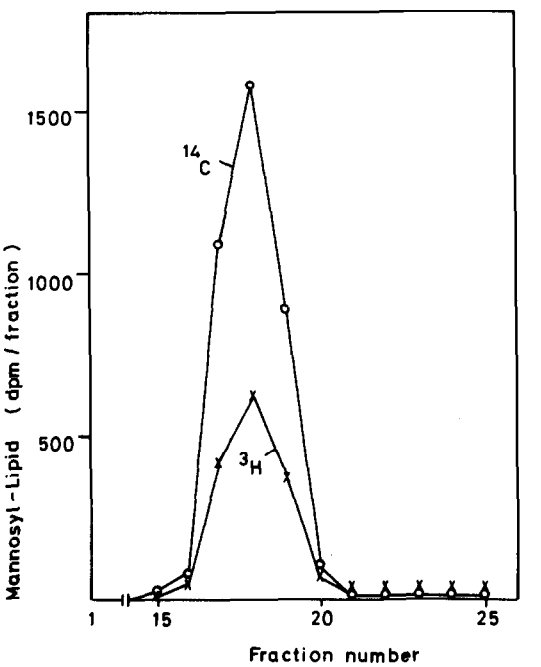

Fig. 1. Chromatography on a Sephadex LH 20 column of the mannosyl-lipid labelled with ^{3}H derived from mevalonic acid in the lipophilic part and with ^{14}C in the mannosyl residue. Segments (10 g) of mung bean shoots were incubated under aeration for 18 hr with 100 μC 5-^{3}H-*DL*-mevalonate. The particles prepared therefrom were incubated with GDP-^{14}C-mannose for 2 min (same as fig. 2), lipids extracted with water-sat. butanol and back-washed with water. The mannosyl-lipid was purified by subsequent chromatography on columns of DEAE-cellulose, silic acid and Sephadex LH 20 (50 X 0.7 cm) [5].

Table 1

Transfer of ^{14}C-mannosyl groups from the mannosyl-lipid to guanosine-diphosphate. The incubation mixture contained 30 µl of buffer [7], 5 µl 0.5 M $MgCl_2$, 5 µl sat. NaF (to inhibit at least partially phosphatases which otherwise rapidly destroy GDP), 20 µl particulate enzyme suspension, 5 µl water or 0.1 M GDP or 0.1 M GMP in water and 6000 cpm ^{14}C-mannosyl-lipid (0.03 mµmoles, isolated in a manner similar to fig. 1). After incubation for 5 min 50 µl acetic acid were added. GDP-mannose was added as carrier and the GDP-^{14}C-mannose and mannosyl-lipid were isolated by chromatography of the total mixture as in fig. 2. The GDP-mannose was further purified by chromatography in isobutyric acid/conc. NH_4OH/water = 57/4/39. Its identity was confirmed by paper electrophoresis in 0.2 M ammonium formiate, pH 3.6, cochromatography in ethanol/0.5 M ammonium acetate, pH 7.5 = 5/2 and by the formation of mannose-1-phosphate on incubation with snake venom phosphodiesterase.

Condition of enzyme	Acceptor present	Lipid recovered (cpm)	GDP-^{14}C-mannose found (cpm)
Boiled	GDP	4116	1
Active	Water	5041	3
Active	GMP	4300	68
Active	GDP	3311	605

tracted from the incubation mixtures with water saturated butanol and was purified by column chromatography as indicated in the legend of fig. 1. Its behaviour during chromatography and its solubility in predominantly alcoholic solutions (98% methanol, 95% ethanol, butanol-pyridinium acetate [8]) but not in pure alcohols as well as its stability against catalytic deacylation [9] showed that the chemical composition of the mannosyl-lipid may be similar to that of the bacterial glycolipid intermediates.

To obtain some more information on the nature of the lipid part, segments of mung bean shoots were incubated in the presence of mevalonic acid-5-^{3}H, the first intermediate of no return on the way to isoprenoid compounds. When the particulate enzyme was prepared from these prelabelled segments and incubated with GDP-^{14}C-mannose, the purified mannosyl-lipid contained both ^{14}C-labelled mannose and ^{3}H derived from mevalonic acid (fig. 1). This can be taken as an indication that the lipid part is of isoprenoid nature. Taking in consideration that polyprenols have been isolated from numerous plants [10] it seems well possible that the lipid part of the mannosyl-lipid described here is a straight chain polyisoprenoid compound rather than a sterol.

3.2. *Reversibility of the synthesis*

The mannose transfer potential of the mannosyl-lipid is evident from three observations. The lipid was fully hydrolyzed with 0.01 N HCl at 100° for 20 min, approximately 90% of the radioactivity being recovered as mannose. The acid lability of the mannosyl residue of the lipid is, therefore, in the same order of magnitude as that of nucleoside diphosphate sugars. Furthermore, if ^{14}C-GDP was added to a reaction mixture under conditions similar to those described in the legend of fig. 2 but containing GDP-^{12}C-mannose, a rapid exchange resulting in the formation of ^{14}C-GDP-mannose was obtained. No similar effect was found with ^{14}C-GMP. When the particles were incubated with GDP-^{14}C-mannose and unlabelled GDP was added after the labelled lipid had reached a steady level, a rapid decrease of ^{14}C-mannose in the glycolipid to less than 10% was obtained. In this case, too, GMP had no effect.

The results of these exchange experiments suggested that the mannosyl residues can be transferred back from the mannosyl-lipid to GDP. This was substantiated by direct experiments in which isolated ^{14}C-mannosyl-lipid was incubated with the particulate enzyme preparation and GDP (table 1). The fact that this resulted in the formation of GDP-^{14}C-mannose shows that the synthesis of the mannosyl-lipid from GDP-mannose occurs by a readily reversible reaction and that, therefore, the group transfer potential of both substances must be in the same order of magnitude.

The mannosyl-lipid described in this communication seems to be similar to that reported recently to occur in yeast [12]. It is dissimilar, however, from the somehow lipophilic material which is formed

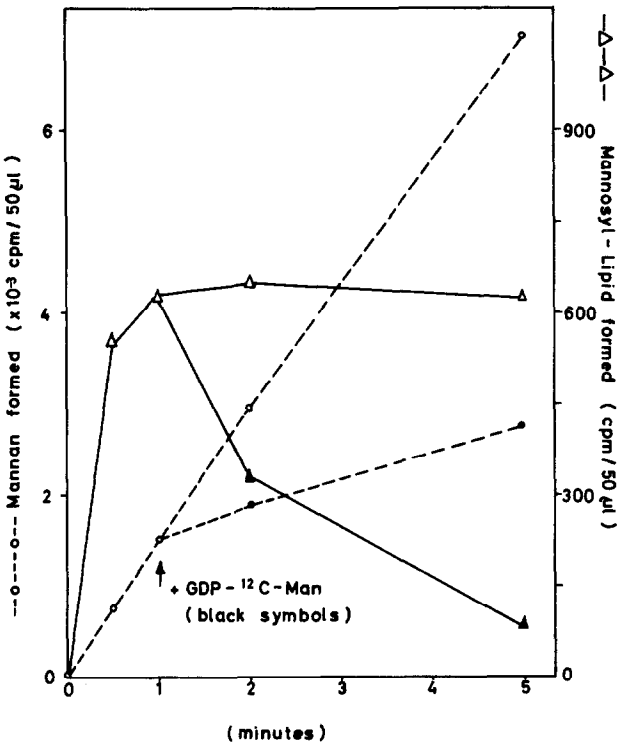

Fig. 2. Chase experiment which may indicate a turnover of the ^{14}C-mannosyl residues of the mannosyl-lipid. The incubation mixture contained 20 µl buffer [7], 5 µl GDP-^{14}-C-mannose (31 400 cpm, 0.16 mµmoles), 5 µl 5 X 10^{-2} M $MgCl_2$, 5 µl 10^{-2} M $MnCl_2$, 5 µl water and 5 µl particulate enzyme suspension [7]. For the chase 5 µl GDP-^{12}C-mannose (15 mµmoles, 5 min incubated with alkaline phosphatase to destroy any GDP) was added. The reactions were stopped by the addition of 50 µl acetic acid and the mixture chromatographed for 5 hr with ethylacetate/butanol/water/acetic acid = 3/4/4/2.5. The material behind the front represents the glycolipid. Most of this material is identical with the glycolipid extracted with saturated butanol as it appeared in the same fractions on chromatography with the three different columns used in fig. 1. The starting line was washed for 18 hr with 10^{-3} M EDTA and counted [7] to give "mannan". This material does not represent unreacted GDP-mannose but a polymer as on hydrolysis with 0.01 N HCl for 20 min at 100° only 2% of the radioactivity was liberated as mannose. By partial hydrolysis with 0.5 ml of fuming HCl + 0.5 ml of conc. HCl (25°, 2 hr) mannose and a series of oligosaccharides were formed. The oligosaccharide behaving like a disaccharide was reduced with sodium borohydride and further hydrolyzed to give a mixture of mannose (54%) and mannitol (46%). This shows that at least two subsequent mannose residues were transferred to a polymer. Part of the mannans synthesized from GDP-mannose by a similar particle preparation have been shown to be an alkali-insoluble glucomannan [11].

from GDP-mannose in mung bean particles and which contains acid-labile mannose-oligosaccharides [13]. The latter can be solubilized only by 45% phenol.

3.3. *Physiological significance*

To demonstrate the physiological significance of the mannosyl-lipid turnover experiments were performed. Fig. 2.shows that the pool of the mannosyl-lipid is rapidly saturated with ^{14}C-mannose, whereas the synthesis of mannan is proportional to time. Addition of nonradioactive GDP-mannose to the reaction mixture at a time when the lipid pool is saturated with ^{14}C-mannose results in a rapid decrease of radioactivity of the lipid, indicating turnover of the mannosyl residues.

One is tempted to speculate that the mannosyl groups disappearing from the glycolipid are transferred to form the polysaccharide which is simultaneously synthesized. This would mean that the plant mannosyl-lipid is an intermediate in the formation of mannan. Such a role has been definitely shown for a similar glycolipid in *Micrococcus* [5,14]. This assumption, however, is not decisive for the plant mannosyl-lipid described here as the apparent turnover may be a manifestation of an exchange occurring with GDP-mannose in a manner similar to the exchange found with GDP.

Experiments undertaken to demonstrate a direct transfer of the mannosyl groups from the isolated ^{14}C-mannosyl-lipid to polysaccharides gave only partial success up to now. Although 50 to 100 cpm of radioactivity were definitely incorporated by a time-dependent reaction, this amount of activity was not enough for detailed identification of the product. The problem in regard to the acceptor molecules for the mannosyl groups of the mannosyl-lipid has, therefore, to await better methods for the reconstitution of the complex membrane system.

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References

[1] A.Wright, M.Dankert, P.Fennesy and P.W.Robbins, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1798.
[2] J.S.Anderson, M.Matsuhashi, M.A.Haskin and J.L. Strominger, Proc. Natl. Acad. Sci. U.S. 53 (1965) 851.
[3] A.Wright, Federation Proc. 28 (1969) 658.
[4] Y.Higashi, J.L.Strominger and C.C.Sweeley, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1878.
[5] M.Scher, W.J.Lennarz, C.C.Sweeley, Proc. Natl. Acad. Sci. U.S. 59 (1968) 1313.
[6] M.G.Heydanek and F.C.Neuhaus, Biochemistry 8 (1969) 1474.
[7] H.Kauss and A.L.Swanson, Z. Naturforsch. 24b (1969) 28.
[8] J.S.Anderson and J.L.Strominger, Biochem. Biophys. Res. Commun. 21 (1965) 516.
[9] A.R.Tarlov and E.P.Kennedy, J. Biol. Chem. 240 (1965) 49.
[10] F.W.Hemming, Biochem. J. 113 (1969) 23P.
[11] A.D.Elbein, J. Biol. Chem. 224 (1969) 1608.
[12] W.Tanner, Biochem. Biophys. Res. Commun. 35 (1969) 144.
[13] C.L.Villemez and A.F.Clark, Biochem. Biophys. Res. Com. mun. 36 (1969) 57.
[14] M.Scher and W.J.Lennarz, J. Biol. Chem. 244 (1969) 2777.