DOLICHOLMONOPHOSPHATES: MANNOSYL ACCEPTORS IN A PARTICULATE *IN VITRO* SYSTEM OF *S. CEREVISIAE*

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1. Introduction

Undecaprenols have been shown to play a major role in the synthesis of cell wall polysaccharides in bacteria [1–4]. For eucaryotic cells the role of "lipids" as intermediates in the biosynthesis of polysaccharides and possibly glycoproteins has also been established [5–10].

Evidence that in liver a dolichol-monophosphate is identical to the "lipid" component of the intermediate has been presented by Behrens and Leloir [9]. Since yeast cells also contain polyprenols of the dolichol family [11] it has been considered possible that the lipid intermediate in yeast mannan biosynthesis described previously [5] is a mannosyl-dolicholphosphate. To test this hypothesis dolicholphosphates from yeast as well as from liver were tested as possible mannosyl acceptors in the yeast system. It will be shown that both can serve as acceptors for the mannosyl residue of GDP-mannose.

2. Material and methods

The particulate fraction of *S. cerevisiae* (strain 66.24, Fleischmann Lab.) was prepared as described previously [5] with minor changes, which include the use of the Bio-X-press for breaking the cells and of tris HCl buffer pH 7.4. Liver dolichol monophosphate was isolated as described [9] and free dolichol from yeast was obtained following the procedure of Burgos et al. [12]. The yeast dolichol was phosphorylated according to Popják et al. [13]. Enzyme test: in a total volume of 70 μl 0.05 M tris HCl pH 7.4 and 60–120 μg protein were incubated with 0.05 μC GDP-mannose-^{14}C (s.A. 154 μC/μmole), 0.25 μmole $MgCl_2$ and 0.025 μmole mercaptoethanol at 22°. Where indicated dolichol-monophosphate corresponding to 6 nmole were added. The reaction was terminated and the lipid extracted as described [9]. The lipid extract was poured into a scintillation vial and dried; Dioxan/PPO was added and the radioactivity determined in a Beckmann LS 100 scintillation counter.

3. Results

The DMP* isolated from liver is glycosylated by a microsomal fraction from rat liver, when sugar nucleotides are added as glycosyl-donors [9, 14]. When this liver DMP is incubated with GDP-mannose-^{14}C and a particulate fraction synthesizing mannan [5] from yeast cells, a strong stimulation of incorporation of radioactivity into the lipid fraction is observed (fig. 1).

* Abbreviations used: DMP = dolichol-monophosphate.

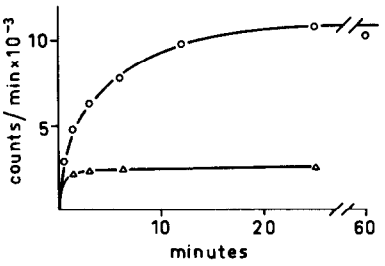

Fig. 1. Incorporation of mannose-^{14}C from GDP-mannose-^{14}C into the lipid fraction. Conditions as given in materials and methods except that EDTA/Mg (2 μmole) were present. △——△ control, ○——○ DMP from liver present.

Table 1
Acid lability of the mannosyl-lipids.

Experiment	counts/min incorporated during 10 min incubation with GDP-mannose-^{14}C	counts/min after acid treatment[a]
I. - DMP	1122	77
+ DMP-liver	4154	148
II. - DMP	5131	255
+ DMP-yeast	10060	359

[a] Conditions see text.

Table 2
Requirements for maximal stimulation of ^{14}C-mannose incorporation from GDP-mannose-^{14}C into the lipid fraction.

Additions	Counts/min incorporated in 10 min	
	- DMP	+ DMP-yeast
None	3916	5286
Triton X-100 (10 μl 0.6%)	3944	6774
EDTA/Mg (2 μmole)	4880	9592
EDTA/Mg + Triton X-100	4317	19903

The additional radioactivity due to the presence of DMP is increasing with time and is almost fivefold that of the control value after 25 min. With Triton X-100 the incorporation in the presence of DMP was about 20% greater; Triton had no effect on the radioactivity of the endogenous acceptor.

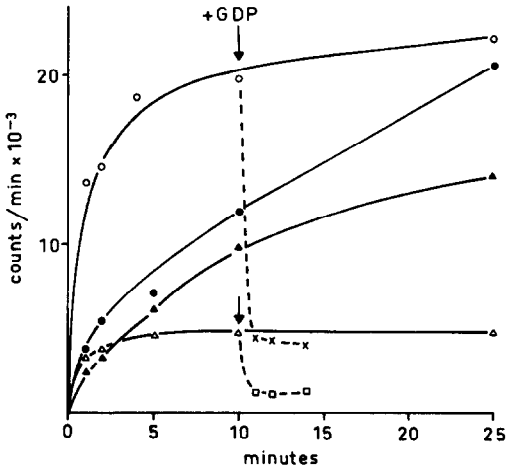

Fig. 2. Incorporation of mannose-^{14}C from GDP-mannose-^{14}C into the lipid fraction and into the non-ethanol soluble residue. Conditions as given in material and methods except that EDTA/Mg (2 μmole) and Triton X-100 (10 μl 0.6%) were present. △——△ lipid fraction control, ○——○ lipid fraction in the presence of DMP-yeast, ▲——▲ residue control, ●——● residue in the presence of DMP-yeast.

The mannosyl linkage of the mannosyl – DMP is acid labile as is the corresponding link in the endogenous lipid (table 1, exp. I); hydrolysis with 1 N HCl at 20° was normally carried out for 1 hr, but 85% of the mannosyl-lipid was hydrolyzed within 15 min.

The dolichols of yeast cells are in the average 3–4 isoprene units shorter than the dolichols of liver [11]. Yeast dolichols were isolated and chemically phosphorylated (see sect. 2). Fig. 2 shows that DMP of yeast prepared in this way also stimulates considerably the incorporation of ^{14}C-mannose into the lipophilic fraction. Again the radioactivity is lost by mild acid treatment (table 1).

Table 2 shows that for optimal stimulation by yeast-DMP Triton X-100 as well as EDTA (Mg-salt) has to be present. These conditions are not optimal for mannan synthesis, since Mn^{2+}-ions are necessary for the transfer of the mannosyl residue from the lipid intermediate to the mannan [5]. In spite of this it can be seen from fig. 2 that considerable radioactivity is incorporated into the alcohol insoluble residue and also that in the presence of DMP this incorporation is enhanced.

Table 3
Reversibility of the reaction.

Experiment	Additions after preincubation[a]	– DMP	+ DMP-liver
I.	None	4474	7308
	GMP (0.1 μmole)	3581	6618
	GDP (0.1 μmole)	711	1518
II.	None	2345	4440
	GDP (0.1 μmole)	654	1398
	GDP (0.1 μmole) (+ acid treatment)	91	102

[a] The preincubation consisted of the normal test (see material and methods) for 20 min. After the addition of GMP or GDP the reaction proceeded for another 2 min.

It has been shown previously [5] that the addition of GDP to the reaction mixture causes a rapid drop in the radioactivity of the lipid fraction and the formation of GDP-mannose-^{14}C; thus the reaction is freely reversible with the endogenous lipid. That this is also the case for the mannosylated DMP from liver and yeast is shown in fig. 2 and table 3. As with the endogenous lipid the loss in radioactivity is only observed when GDP and not when GMP is added. This indicates a phosphodiester link in the DMP-mannose analogous to the DMP-glucose described by Behrens and Leloir [9] and the undecaprenol-mannose described by Scher, Lennarz and Sweeley [3] but different from the pyrophosphate bond of other bacterial lipid-intermediates [1, 2, 4].

The drop in radioactivity of the lipid is very fast; in less than 2 min the back reaction is finished. The residual radioactivity remaining in the lipid (fig. 2, table 3) is still acid labile (exp. 2 in table 2); possibly an equilibrium situation is reached within two min. It is noteworthy that the rate of the back reaction for the endogenous lipid seems to be as fast as that for the exogenous DMP, which might indicate that the mannosylated DMP is fully integrated into the membrane. In the time course of the forward reaction on the other hand a slower phase shows up (fig. 1) which might be caused by a rate limiting integration of free DMP into the membrane.

That the mannosyl-DMP produced in this yeast in vitro system is indeed an intermediate in mannan biosynthesis is so far only indicated by a slight but significant stimulation of ^{14}C-mannose incorporation into an alcohol insoluble polymer. The proposed transfer of mannose from DMP-mannose to the growing mannan chain is presently under investigation.

The results reported show that dolicholphosphates serve well as mannosyl acceptors in the particulate system of yeast. For the reaction exists no strict specificity as far as the chain length of the dolichols used is concerned (yeast versus liver). This is in agreement with a short report of Alam et al. [15] who have shown recently that quite a variety of polyprenolphosphates can serve as mannosyl acceptors in pig liver as well as in mung bean preparations.

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