BIOSYNTHESIS OF LIPID-LINKED SUGARS IN *SACCHAROMYCES CEREVISIAE*

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Received 7 May 1973

1. Introduction

The role of polyprenyl phosphates in the biosynthesis of various sugar polymers has been demonstrated in bacterial as well as eukaryotic cells. Tanner [1] presented evidence that a lipophilic mannose derivative might be the precursor of yeast mannan and later [2] observed a moderate stimulation of the formation of the mannolipid on using chemically phosphorylated dolichols from pig liver (a mixture of cis/trans C_{85}—C_{110} polyprenols with a saturated α-residues) and from yeast (a mixture of cis/trans C_{70}—C_{90} polyprenols with saturated α-residues). The formation of the mannolipid by particulate enzyme preparations from yeast as reported by Sentandreu and Lampen [3, 4] was the intermediate step in the biosynthesis of mannan from GDP-mannose.

The present paper describes the formation of lipid-linked sugars by particulate enzyme preparation from yeast using UDP-glucose and UDP-galactose as the sugar donors. The reaction of these nucleoside diphosphate sugars and of GDP-mannose with both endogenous lipid acceptors from yeast and from pig liver as well as with the monophosphate of the plant polyprenol—ficaprenol (a mixture of C_{50}-, C_{55}- and C_{60}-cis/trans polyprenols [5] is the subject of this report.

2. Materials and methods

GDP-[C_{14}]mannose and UDP-[^{14}C]galactose were from The Radiochemical Centre, Amersham, Bucks., England. UDP-[^{14}C]glucose was prepared by the method of Wright and Robbins [6]. [β-^{32}P]UDP-glucose and ficaprenyl phosphate were the same as in the previous studies [7]. Unlabelled nucleoside diphosphate sugars were from Serva, Heidelberg, Germany.

A commercial yeast strain, cultivated on a medium containing 2% glucose, 2% peptone and 2% yeast extract was collected in early stationary phase (second generation) and used routinely. Two wild strains from our own collection (S-288C, haploid and SBTD, diploid) were also tested.

Enzyme preparation: Fresh yeast cells were mechanically disrupted with Ballotini beads, suspended in Tris-maleate buffer (50 mM), pH 7, containing 3 mM mercaptoethanol and centrifuged at 1000 *g* and 10 000 *g* for 10 min and the sediment was discarded. The supernatant was centrifuged at 105 000 *g* for 1 hr and the resulting pellet of particulate fraction was used as the source of enzyme.

Lipid acceptor from yeast (crude dolichol phosphate preparation) was obtained by the method of preparation of dolichol phosphate from pig liver [7, 8]. Lipid extract obtained from 1 kg of fresh baker's yeast (partially dried and defatted with acetone) was subjected to alkaline and acid hydrolysis and fractionated on the column of DEAE-cellulose with increasing concentrations of ammonium acetate (0–0.2 M) in chloroform—methanol, 2:1 mixture. The fractions were washed with water to remove ammonium acetate [9] and tested for the presence of sugar-binding lipids and for total phosphorus [10].

The biosynthesis of lipid-linked sugars was measured after 1 hr of incubation of enzyme preparation with the appropriate lipids and labelled nucleoside diphosphate sugars as described in a previous paper [7].

3. Results and discussion

The yeast cells contain an alkali- and acid-stable phospholipid which is eluted from DEAE-cellulose in approximately the same position as dolichol phosphate from pig liver [7], and stimulates the incorporation of glucose from UDP-glucose into the lipid fraction 5–7 times in the presence of rat liver microsomes. This effect is similar to that of dolichol phosphate and ficaprenyl phosphate that was described earlier [11]. The addition of ficaprenyl phosphate greatly increases the incorporation of sugars from nucleoside diphosphate sugars into lipids by enzyme preparation from yeast (table 1). GDP-mannose was the most effective precursor in this reaction, but with UDP-glucose and UDP-galactose the formation of lipid-linked sugars was also observed. The observed state of this reaction is higly dependent on the yeast strain. For example, commercially obtained yeast gave higher yield of total enzyme units than yeast from our own collection probably because the latter yeast (as we found) has a higher level of enzymes which degrade both nucleoside diphosphate sugars and prenyl phosphates.

The formation of lipid-linked glucose was linear during 1 hr of incubation and after 2 hr slightly decreased (fig. 1). The enzyme preparation was active in a wide range of pH with the optimum in pH 7.0 (fig. 2). The magnesium salt of EDTA was necessary for full enzyme activity, was better than $MgCl_2$ alone, and could not be replaced by Mn^{2+} ions.

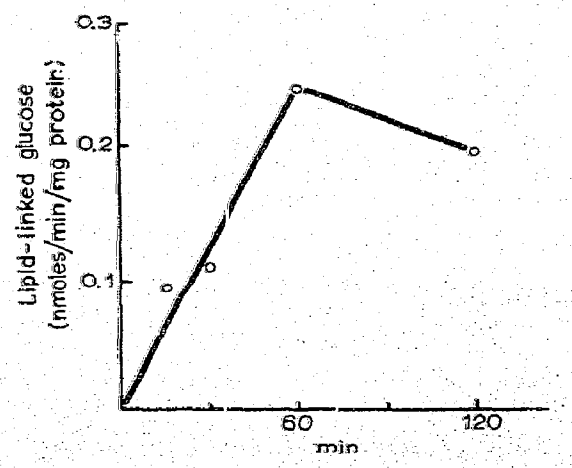

Fig. 1. Incorporation of glucose from UDP-[^{14}C]glucose into the lipid fraction. Effect of time of incubation. Conditions as given in table 1.

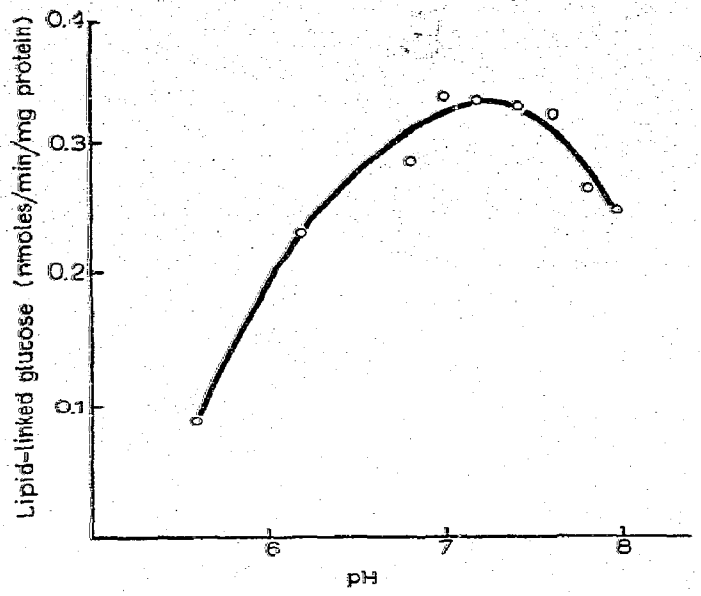

Fig. 2. Effect of pH on the incorporation of glucose from UDP-[^{14}C]glucose into the lipid fraction. Conditions as given in table 1.

The amount of sugar transferred to the lipid fraction is dependent on the amount of lipid acceptor added (fig. 3). A rather high level of ficaprenyl phosphate is necessary for full activity of the enzyme. This may be due to the presence of polyprenyl phosphate phosphatase in yeast (G. Palamarczyk, unpublished results).

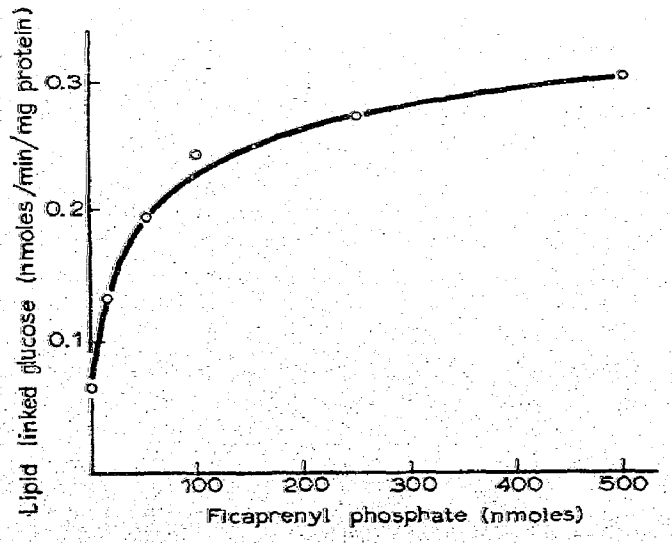

Fig. 3. Effect of ficaprenyl phosphate concentration on the incorporation of glucose from UDP-[^{14}C]glucose into the lipid fraction. Conditions as given in table 1.

Table 1
Effect of ficaprenyl phosphate on the formation of lipid-linked sugars.

Nucleoside diphosphate sugar	Ficaprenyl phosphate	(pmoles of lipid-linked hexose/min/mg protein)
UDP-glucose	–	3
	+	80
UDP-galactose	–	4
	+	20
GDP-mannose	–	14
	+	467

The incubation mixture contained in a final vol of 100 µl: 100 nmol of ficaprenyl phosphate, 1 µmol of Mg_2EDTA, 12 µmol of Tris–maleate buffer, pH 7.0, 40 nmol of mercaptoethanol, 250 nmol of the indicated nucleoside diphosphate [^{14}C]sugar, 0.6% Triton X-100 and 370 µg of enzyme protein (standard incubation conditions).

The incorporation of glucose from UDP-glucose and mannose from GDP-mannose into lipid fraction was also stimulated by naturally occurring dolichol phosphates from pig liver and from yeast (table 2). However, the effect of these phospholipids can not be compared with that of ficaprenyl phosphate as the exact amount of dolichol phosphate in crude preparations obtained from pig liver and yeast is not shown. The stimulating effect of the preparation of dolichol phosphate from yeast was similar to that observed by Tanner et al. [2] but the high stimulation observed with dolichol phosphate from pig liver was rather surprising.

Table 2
Effect of crude dolichol phosphate preparations (Dol-P) from pig liver and yeast on the formation of lipid-linked sugars.

Nucleoside diphosphate sugar	Crude Dol-P preparation	(pmoles of lipid-linked hexose/min/mg protein)
UDP-glucose	None	3
UDP-glucose	Dol-P from pig liver	6
UDP-glucose	Dol-P from yeast	14
GDP-mannose	None	8
GDP-mannose	Dol-P from pig liver	275
GDP-mannose	Dol-P from yeast	23

The incubation was done in the standard conditions except that 130 µg of enzyme protein was used and ficaprenyl phosphate was replaced by crude dolichol phosphate preparation from pig liver (50 nmol of lipid P) and from yeast (10 nmoles of lipid P).

When [β-^{32}P]UDP-glucose was used as the sugar donor, the radioactivity was not incorporated into lipids. This result points to a phosphoryl rather than pyrophosphoryl linkage of glucose to the lipid. The same type of structure was suggested for the yeast mannolipid studied by Sentandreu and Lampen [4].

As shown in this paper the biosynthesis of lipid-linked sugars in yeast may be involved not only in the transfer of mannosyl residue in the process of the formation of mannan. The results obtained point to a more general character of polyprenyl phosphate sugars in yeast and at a relative unspecificity of the enzyme lipid-linked sugars with respect to the type of lipid acceptor.

Acknowledgements

We thank Dr. J. Rytka and Dr. A. Putrament for yeast strains and helpful discussion. This work was supported by the Polish Academy of Sciences within the project 09.3.1.1.1.9.

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