POLYPRENYL PHOSPHATE PREVENTS INACTIVATION OF YEAST GLYCOSYL TRANSFERASE BY DETERGENTS

G. PALAMARCZYK, L. LEHLE and W. TANNER
Fakultät für Biologie und Vorklinische Medizin, Universität Regensburg, 8400 Regensburg, FRG

Received 26 September 1979

1. Introduction

Although the importance of the dolichol pathway in forming carbohydrate portions of glycoproteins is generally accepted [1–4], the membrane-bound enzymes involved in these reactions are so far not studied in any detail. The main obstacles for such investigations are the solubilization and stabilization of the proteins in question as well as the need for a test system with defined substrates for the solubilized enzymes. A solution of the latter problem seems in sight, since well-defined polyprenols [5] as well as small peptides which serve as carbohydrate accepting units [6–8] are now available.

Here we describe a strong inhibitory effect of non-ionic detergents on the following glycosyl transfer reactions:

UDP-GlcNAc + Dol-P → Dol-P-P-GlcNAc + UMP (I)

Dol-P-P-GlcNAc + UDP-GlcNAc → Dol-P-P-$(GlcNAc)_2$ + UDP (II)

GDP-Man + Dol-P → Dol-P-Man + GDP (III)

This inhibitory effect can be prevented by polyprenyl phosphates. The specificity and concentration dependence of the protective agents suggest that the active site of the enzymes must be occupied by the substrate to keep the enzymes in a form which is non-susceptible to detergents.

2. Materials and methods

The membrane fraction was prepared from *Saccharomyces cerevisiae* (strain 66.24, Fleischmann Labs.) as in [9]. Preincubation of the enzyme with detergents was carried out routinely over 30 min at 0°C in a medium containing: $MgCl_2$ (10 mM); Tris–HCl (pH 7.5) (40 mM); detergent (0.15% or 0.2% to show the effect on mannosyl transfer); yeast membranes (75–100 μg protein) in 50 μl total vol. Membranes preincubated in an identical way except for the presence of detergent were used as a control. Polyprenylphosphate (5 nmol single chain-length C_{100}- or C_{55}-dolichylphosphates) as well as the other lipids tested were introduced to the preincubation medium after drying them under nitrogen or drying them together with 2 μmol MgEDTA (where indicated).

Various polyprenylphosphates [5] were obtained from Dr T. C. Chojnacki (Polskie Odczynniki Chem.).

Transfer of *N*-acetylglucosamine from UDPGlcNAc to polyprenylphosphate was measured over 30 min at 23°C in the medium containing: 6 μmol C_{100}-dolichylphosphate dried together with 2 μmol MgEDTA, $MgCl_2$ (8.3 mM), Tris–HCl (pH 7.5) (33 mM), Nonidet P-40 or other detergents tested at 0.125% final conc., UDPGlcNAc (0.1 μCi, spec. act. 300 Ci/mol) preincubated membrane preparation 80–100 μg protein) in 60 μl total vol.

Formation of DolPMan was measured over 5 min at 23°C as in [9].

Labelled lipids were extracted and washed as in [9]. Thin-layer chromatography was performed on Merck Silica GelG [10].

3. Results and discussion

N-Acetylglucosamine transferases have been solubilized from porcine aorta by Nonidet, and to a lesser extent by Triton [11]. These detergents, when added to the incubation mixture with yeast membranes inhibit the transfer of [^{14}C]GlcNAc from UDP-[^{14}C]-GlcNAc to endogenous lipid considerably (fig.1). In the presence of detergent and of exogenous dolichylphosphates, however, the activity is maintained and at 0.15% detergent even a stimulation of ~20% as compared with membranes in the absence of detergent and exogenous dolichylphosphates is observed. The inhibitory effect at low concentrations of the detergents most likely is due to solubilizing the endogenous dolichylphosphate, thereby lowering the actual lipid acceptor concentration in the vicinity of the enzymes.

Under the experimental conditions of fig.1 the exogenously supplied dolichylphosphate indeed acts as acceptor for GlcNAc; this is shown in fig.2. In this

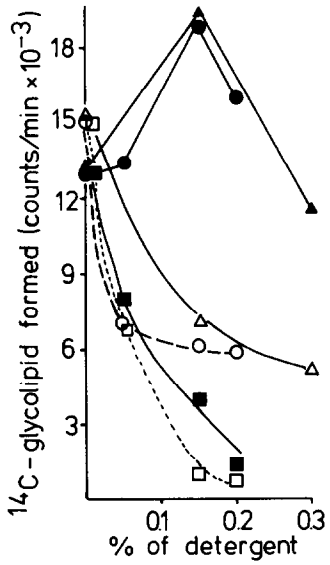

Fig.1. Effect of detergents on the GlcNAc transferase activity of yeast membranes. The transfer of radioactivity from UDP-[^{14}C]GlcNAc into the total lipid soluble fraction is plotted. Open symbols transfer activity to endogenous lipid acceptor; closed symbols transfer activity in the presence of 5 nmol C_{100}-dolichylphosphate. For other conditions see section 2. (○, ●) Triton X-100; (△, ▲) Nonidet P40; (□, ■) deoxycholate.

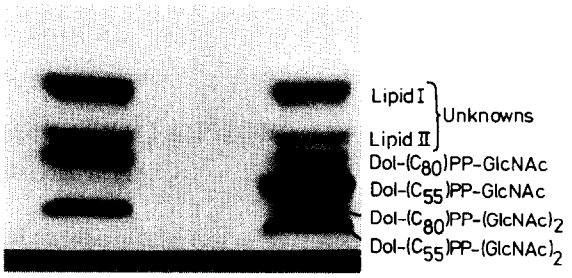

Fig.2. GlcNAc transfer to exogenously supplied C_{55}-dolichylphosphate. (A) Control incubation in the presence of 0.15% Triton X-100; for other conditions see section 2. (B) as (A) plus 5 nmol C_{55}-dolichylphosphate.

experiment C_{55}-dolichylphosphate was added as exogenous acceptor and the mono- and disaccharide formed with the polyprenylphosphate separates on thin layer from those formed with the endogenous C_{80}-dolichylphosphate.

During the course of studies undertaken to solubilize the GlcNAc transferases it has been observed that the inhibitory effect of Nonidet and of Triton cannot be overcome anymore, when polyprenylphosphates are added a few minutes after the detergents. Thus, besides lowering the endogenous dolichylphosphate concentration by detergents, the latter also seem to irreversibly inactivate the enzyme in the absence of its substrate. This effect is clearly shown in table 1. A preincubation of membranes in the presence of Nonidet or Triton at 0°C for 30 min (however 3 min are already sufficient for the effect) leads to a loss of enzyme activity of ⩽90%, although exogenous C_{100}-dolichylphosphate was present during the determination of enzyme activity, i.e., after the preincubation. The radioactivity given in table 1 and for the following experiments refers to the transfer of GlcNAc to exogenous polyprenylphosphates only; transfer to all endogenous acceptors has been subtracted. If the preincubation is carried out in presence of C_{100}-dolichylphosphate, only ~10–20% of the enzyme activity is lost. The same small degree of inactivation is observed when the preincubation in the presence of prenylphosphate is extended ⩽3 h. With deoxycholate the preincubation effect is not observed, however with this detergent even without preincubation only 1/3rd of the enzyme activity is measurable (table 1).

Table 1
Effect of detergents on the activity of UDP-GlcNAc → polyprenylphosphate transferase

Treatment	Detergent present during pretreatment and test[a]		
	Nonidet P40	Triton X-100	Na-deoxycholate
	[^{14}C]Glycolipid formed (cpm . 80 μg protein^{-1} . 30 min^{-1})[b]		
No pretreatment (control)	23 438	24 144	6336
Pretreatment with detergent (0°C, 30 min)	2530	2270	7238
Pretreatment with detergent plus C_{100}-dolichylphosphate + Mg EDTA[c] (0°C, 30 min)	20 560	19 099	8765

[a] 0.8 mg/mg protein
[b] The two unknown radioactive lipids (I) and (II) (see also fig.2) amount to ~5% of the total radioactivity in the control. The pretreatment (with Nonidet or Triton) does not change the radioactivity in these two lipids
[c] 5 nmol C_{100}-dolichylphosphate, 2 μmol Mg-EDTA

The protective effect by polyprenylphosphate was much lower at room temperature (only ~25%) than at 0°C. On the other hand, the irreversible inactivation by detergents is a function of the protein/detergent ratio and is lower at ratios >1.

The protection by polyprenyl phosphates against irreversible detergent inactivation is a very specific effect. As shown in table 2 neither the α-unsaturated C_{100}-polyprenylphosphate, which also does not serve as a substrate for this enzyme [9], nor free yeast dolichol (mainly C_{80}), nor lecithin is able to protect to a significant extent. C_{100}-Dolichylphosphate yields

Table 2
Protection of the GlcNAc transferase activity against Nonidet P40 inactivation

Present during pretreatment (0°C, 30 min)			[^{14}C]Glycolipid formed (cpm . 40 μg protein^{-1} . 30 min^{-1})
Nonidet P40	Mg-EDTA	'Protecting' agent	
–	–	–	10 686
+	–	–	294 (3%)
+	+	C_{100}-Dolichylphosphate	9404 (88%)
+	–	C_{100}-Dolichylphosphate	5703 (53%)
+	+	–	791 (7%)
+	+	C_{100}-polyprenylphosphate (α-unsaturated)	2000 (19%)
+	–	C_{100}-polyprenylphosphate (α-unsaturated)	652 (6%)
+	+	Yeast dolichol	1467 (14%)
+	–	Yeast dolichol	753 (7%)
+	+	Lecithin	2374 (22%)
+	–	Lecithin	690 (6%)
+	–	UDP-GlcNAc	2137 (20%)

an almost complete protection if also Mg-EDTA is present (Mg^{2+} alone has no effect, whereas EDTA alone strongly inhibits the reaction). The positive effect of Mg-EDTA on enzymes transfering sugars to exogenous dolichylphosphates has been known for a long time [12,13]. UDP-GlcNAc, the other substrate of the enzymes, shows only little protective action.

The results reported suggest that the active site for prenylphosphates of the GlcNAc-transferases has to be occupied so that the enzymes are not susceptible towards detergents. For the enzyme catalyzing reaction (II) (see above) actually Dol-P-P-GlcNAc should be the corresponding substrate, however the dolichylphosphate part might already suffice to bring about the protection. It cannot be excluded on the other hand that only the enzyme catalyzing reaction (I) is affected by the detergent as well as by the protecting dolichylphosphate, since an inhibition of reaction (I) will also completely prevent the formation of the lipid disaccharide [10].

When the dependence of the protective effect on the concentration of prenylphosphates is measured a saturation curve is obtained with an app. K_m 4×10^{-5} M. This value agrees very well with the K_m for polyprenylphosphate when it acts as GlcNAc acceptor in the actual transfer reaction (fig.3). The argument is strengthened, therefore, that polyprenylphosphates have to occupy the enzyme active site in order to act as protectors against detergents.

Also the enzyme catalyzing the mannosylation of Dol-P to Dol-P-Man is affected by detergents in a similar way to the GlcNAc transferases and can also be protected by prenylphosphates (table 3).

Table 3
Effect of Nonidet P-40 on the activity of GDP-Man → dolichylphosphate transferase

Treatment	Dolichylphosphate mannose formed (cpm . 75 μg protein^{-1} . 5 min^{-1})
No pretreatment (control)	32 122
Pretreatment with Nonidet (0.8 mg/mg protein) 0°C, 30 min	7125
Pretreatment with Nonidet + C_{100}-dolichylphosphate (5 nmol)	73 827

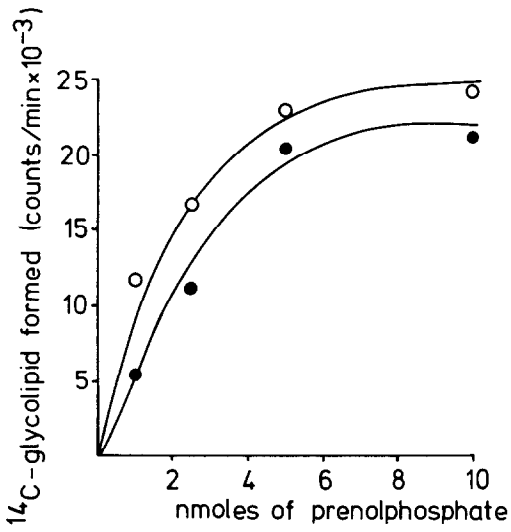

Fig.3. The dependence of the protective effect on the concentration of prenylphosphate. (•——•) The membranes have been preincubated with Nonidet P40 according to table 1 and section 2. During preincubation increasing amounts of C_{100}-dolichylphosphate as indicated were present; during the test saturating amounts of prenylphosphate (10 nmol) were present. (○——○) The GlcNAc transferase activity of the membranes was tested without preincubation. The test was carried out as in section 2 with varying amounts of C_{100}-dolichylphosphate.

The phenomena described can be explained by assuming the existence of two conformational states of the enzymes, only one being highly susceptible towards detergents. The presence of polyprenylphosphate at the active site of the enzyme would have to change the enzyme to a form not susceptible to detergent. Whether such a postulated conformational change of the enzymes might be related to their possible function of translocating sugars through the membrane can be, of course, merely a matter of speculation in the moment. It also cannot be excluded that the detergent and the prenylphosphate simply compete for the active site. This seems less likely, however, since in this case a reversibly bound substrate should not be able to protect against an irreversible detergent inactivation; the substrate would be expected only to slow down the inactivation process.

Acknowledgement

This work has been supported by a grant of the Deutsche Forschungsgemeinschaft.

References

[1] Waechter, C. J. and Lennarz, W. J. (1976) Ann. Rev. Biochem. 45, 95–112.
[2] Parodi, A. J. and Leloir, L. F. (1979) Biochim. Biophys. Acta 559, 1–37.
[3] Elbein, A. D. (1979) Ann. Rev. Plant Phys. 30, 239–272.
[4] Babczinski, P. and Tanner, W. (1973) Biochem. Biophys. Res. Commun. 54, 1119–1124.
[5] Mankowski, T., Jankowski, W., Chojnacki, T. and Franke, P. (1976) Biochemistry 15, 2125–2130.
[6] Ronin, C., Bouchilloux, S., Granier, C. and Van Rietschoten, J. (1978) FEBS Lett. 96, 179–182.
[7] Struck, D. K., Lennarz, W. J. and Brew, K. (1978) J. Biol. Chem. 253, 5786–5794.
[8] Bause, E. and Lehle, L. (1979) Eur. J. Biochem. in press.
[9] Pless, D. D. and Palamarczyk, G. (1978) Biochim. Biophys. Acta 529, 21–28.
[10] Lehle, L. and Tanner, W. (1976) FEBS Lett. 71, 167–170.
[11] Heifetz, A. and Elbein, A. D. (1977) J. Biol. Chem. 252, 3057–3063.
[12] Behrens, N. H. and Leloir, L. F. (1970) Proc. Natl. Acad. Sci. USA 66, 153–159.
[13] Tanner, W., Jung, P. and Behrens, N. H. (1971) FEBS Lett. 16, 245–248.