

PROTEIN GLYCOSYLATION THROUGH DOLICHOL DERIVATIVES IN BAKER'S YEAST

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1. Introduction

Dolichol-bound sugars are intermediates in the glycosylation of proteins [1–3]. Incubation of liver microsomes with UDP-Glc and Dol-P leads to the synthesis of Dol-P-Glc which in turn may transfer its glucosyl residue to an endogenous acceptor. The glucosylated endogenous acceptor (GEA) is a Dol-PP derivative containing two GlcNAc and probably twelve Man and four Glc residues [4–6]. The synthesis of GEA has been detected in microsomes of rat brain, kidney and liver, pig liver, human lymphocytes and hen oviduct ([5] and H. Carminatti, personal communication). The synthesis of Dol-P-Man and of derivatives containing variable amounts of Man residues joined to *N,N*-diacetylchitobiosyl dolichol pyrophosphate has been found to occur in the microsomes of several animal cells and in plants [2,7,8]. While upon mild acid hydrolysis these derivatives give a series of oligosaccharides due to the different number of Man residues, the glucosyl derivative contains apparently only one oligosaccharide of defined composition. The transfer of the hydrophilic moieties of GEA and of the Dol-PP derivatives containing GlcNAc and Man residues to endogenous proteins has been detected in animal cell microsomes [7,9–11]. The glycoproteins thus

synthesized are probably of the asparagine-linked type because in all cases studied the Man-GlcNAc-GlcNAc-containing oligosaccharides have been found joined to an asparagine residue.

The synthesis of Dol-P-Man and of Dol-PP(GlcNAc)₂-(Man)₂ has been found to occur in yeast [12,13]. The former dolichol derivative is the immediate precursor of the mannosyl residues joined to Ser/Thre in yeast mannan. Palamarczyk and Chojnacki [4] have found that the synthesis of a labeled glycosyl compound soluble in organic solvents by yeast particulate proteins is stimulated by the addition of partially purified liver Dol-P. However, a clear identification of this compound as Dol-P-Glc is lacking.

The wide distribution of GEA among several animal tissues prompted us to look for its synthesis in such a distant phylogenetic species as yeast.

2. Materials and methods

Dol-P-[¹⁴C]Glc and [¹⁴C]GEA were synthesized with rat-liver microsomes as previously described [5,15]. They were kept in chloroform/methanol (2:1) and in chloroform/methanol/water (1:1:0.3) respectively at –20°C. GEA was labeled in the Glc residues.

The yeast particulate fraction was prepared from commercial baker's yeast as described by Lehle and Tanner [16]. It was finally suspended in 50 mM Tris–HCl buffer pH 7.5, 5 mM MgCl₂ and 5 mM mercaptoethanol at a protein concentration of 50 mg/ml and kept at –70°C.

Abbreviations: DOC: sodium deoxycholate; Dol: dolichol; GEA stands for glucosylated endogenous acceptor. It is a Dol-PP derivative containing two GlcNAc and probably twelve Man and four Glc residues

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3. Results and discussion

Incubation of UDP- $[^{14}\text{C}]$ Glc with the yeast particulate fraction led to the synthesis of a compound that was indistinguishable from Dol-P-Glc synthesized by liver microsomes as judged by several analytical criteria. The only difference that could be found between the yeast and liver Dol-P-Glc was the molecular size of the dolichol moieties. According to the molecular weights measured in Sephadex G-75 columns equilibrated with DOC, the yeast compound had 16 isoprene residues instead of the 19 residues found in the liver dolichol derivative. A detailed characterization of yeast Dol-P-Glc will be published elsewhere.

Dol-P-Glc and GEA can be easily distinguished in an incubation mixture because the former is soluble in chloroform/methanol (2:1) and in the lower phase of a chloroform/methanol/water (3:2:1) partition while the latter remains with the protein precipitate. It can be solubilized with chloroform/methanol/water (1:1:0.3). The synthesis of a compound with the solubility properties of GEA was observed when liver Dol-P- $[^{14}\text{C}]$ Glc was incubated with the yeast particulate fraction. The substrate used was that obtained with liver microsomes because Dol-P-Glc was synthesized in poor yield by the yeast particulate fraction. A detergent dependence curve of the transfer reaction from Dol-P-Glc is depicted in fig.1. The optimal DOC concentration appeared to be 0.4% in our experimental conditions. This detergent could not be replaced by Triton X-100. The reaction did not require bivalent cations. A similar result had been obtained with liver microsomes [15]. Only labeled glucose was detected upon mild acid hydrolysis and paper chromatography of the radioactive substance that remained in the lower phase and in the chloroform/methanol (2:1) washings. This substance was therefore, unreacted Dol-P-Glc. The compound soluble in chloroform/methanol/water (1:1:0.3) was decomposed by a mild acid treatment. The label became water-soluble and ran in paper chromatography at the same rate as a maltosaccharide of 16 glucose units, the same mobility as a sample prepared from liver GEA (fig.2). The compounds synthesized from Dol-P-Glc either by liver microsomes or the yeast particulate fraction had the same solubility in organic solvents, were acid-labile and their hydrophilic moieties had the same mobility in paper chromatography. It can be tentatively assumed there-

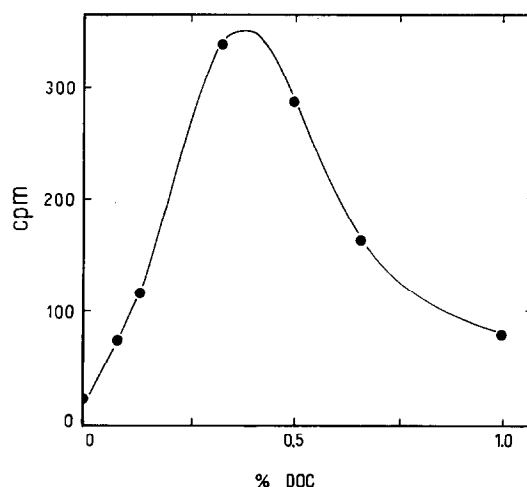


Fig.1. Transfer of Glc residues from Dol-P-Glc by the yeast particulate fraction. The incubation mixtures contained 4000 cpm of liver Dol-P- $[^{14}\text{C}]$ Glc, 70 mM Tris-maleate buffer pH 7.6, 70 mM mercaptoethanol, variable amounts of DOC, 7 mM EDTA and 1 mg of yeast particulate protein in a total volume of 75 μl . The labeled substrate was dried under a nitrogen stream before adding the other components. After 30 min at 30°C the reactions were stopped by the successive addition of 0.4 ml of methanol, 0.15 ml of 4 mM MgCl_2 and 0.6 ml of chloroform. The protein interphases were extracted twice with 1.5 ml of chloroform/methanol (2:1) and twice with 1 ml of chloroform/methanol/water (1:1:0.3). Both water-containing extracts were pooled, dried on planchets and counted in a flow counter.

fore, that both the liver and the yeast compounds have similar structures.

The last reaction in the glycosylation pathway, that is, the transfer of the oligosaccharide moiety from the Dol-PP derivative to an endogenous acceptor protein has been found to occur also in the yeast particulate fraction (fig.3). The substrate used was $[^{14}\text{C}]$ GEA from liver microsomes because it was synthesized in low yield by the yeast particulate fraction. The transfer reaction required bivalent cations, Mn^{2+} being more effective than Mg^{2+} . It has, therefore, the same characteristics as the liver system [11]. The reaction product is probably a glycoprotein, as in liver microsomes, because the label that was initially insoluble in 10% trichloroacetic-acid- became soluble in the same solvent after the trichloroacetic-acid-precipitate was heated in 2 N KOH for 10 min at 100°C. The trichloroacetic-acid-soluble substance was

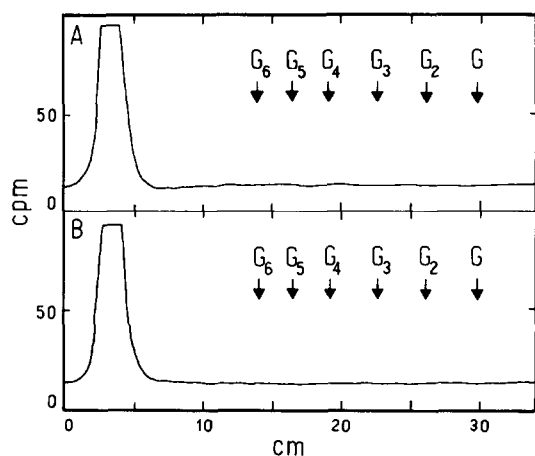


Fig. 2. Paper chromatography of the hydrophilic moieties from yeast and liver GEA. The radioactive compounds soluble in chloroform/methanol/water (1:1:0.3) synthesized from Dol-P- $[^{14}\text{C}]\text{Glc}$ by the yeast particulate fraction (A) or by liver microsomes (B) were dried under a nitrogen stream and heated in aqueous 0.01 M HCl for 5 min at 100°C . A chloroform/methanol/water (3:2:1) partition was then performed and the upper phase was spotted on Whatman No. 1 paper. Glucose and maltosaccharides (G, G_2 , G_3 , etc.) were added as internal standards. Chromatography was performed with 1-butanol/pyridine/water (4:3:4) as solvent. After scanning the paper in a radiochromatogram scanner, the saccharides were revealed according to Trevelyan et al. [17].

charged as judged by the fact that 64% of the label was retained by a mixed bed (Amberlite MB-3) column. The charge was presumably due to amino-acid residues and not to the sugar moiety since the oligosaccharide obtained by acid methanolysis of liver GEA [18], treated with alkali under the same conditions was only partially retained (8%) by the resin. The charge in this compound was probably due to a partial deacetylation of the GlcNAc residues during the alkaline treatment [5].

From the results presented in this paper it can be concluded that the following reactions of Glc transfer, already known to occur in liver microsomes, take place in yeast:

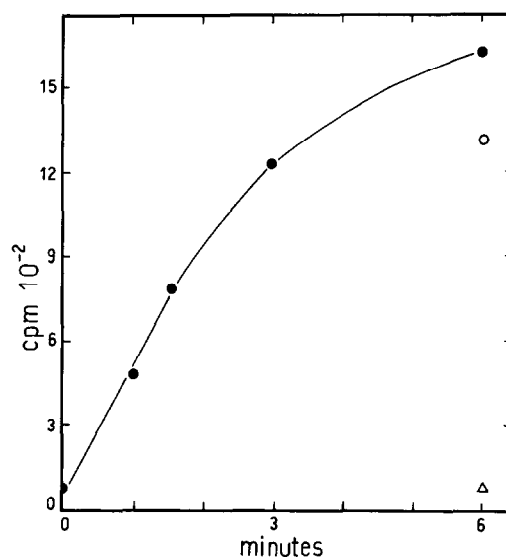
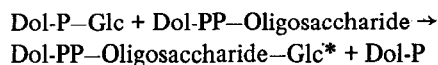
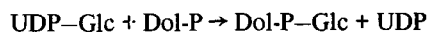


Fig. 3. Transfer of the oligosaccharide moiety from GEA to an endogenous protein by the yeast particulate fraction. The incubation mixtures contained 37 000 cpm of liver $[^{14}\text{C}]\text{GEA}$, 70 mM Tris-maleate buffer pH 7.6, 70 mM mercaptoethanol, 0.8% Triton X-100 and 4.5 mg of yeast particulate protein in a total volume of 450 μl . The incubation mixtures were supplemented with 7 mM MnCl_2 (●) or 7 mM MgCl_2 (○) or 7 mM EDTA (△). The labeled substrate was dried under a nitrogen stream before adding the other components. The incubations were performed at 15°C for the indicated times. Aliquots of 70 μl were withdrawn and heated in 1 ml of 10% trichloroacetic acid at 100°C for 5 min. All GEA is decomposed under these conditions, giving radioactive soluble products. The precipitates were washed twice with 1 ml of 10% trichloroacetic acid, once with 1 ml of diethyl ether, once with 1 ml of methanol, dissolved in Protosol and a toluene-based scintillation mixture and counted in a liquid scintillation spectrometer.

Protein-Oligosaccharide-Glc + Dol-PP.

This is the first demonstration that an oligosaccharide may be transferred from a Dol-PP derivative to yeast endogenous proteins. The fact that the same complex oligosaccharide derivative of dolichol, containing about two GlcNAc, twelve Man and four Glc residues is synthesized in several mammalian tissues, in avians and in yeast suggests that the respective membrane glycoprotein(s) may play a common and important function.

*GEA: Dol-PP-Oligosaccharide-Glc

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References

- [1] Behrens, N. H. (1974) in: *Biology and Chemistry of Eucariotic Cell Surfaces* (Lee, E. Y. C. and Smith, E. E. ed) Vol. 7, pp. 159–178, Academic Press, New York.
- [2] Lennarz, W. J. (1975) *Science* 188, 986–991.
- [3] Parodi, A. J. and Leloir, L. F. (1976) *TIBS* 1, 58–59.
- [4] Parodi, A. J., Behrens, N. H., Leloir, L. F. and Dankert, M. (1972) *Biochim. Biophys. Acta* 270, 529–536.
- [5] Parodi, A. J., Staneloni, R., Cantarella, A. I., Leloir, L. F., Behrens, N. H., Carminatti, H. and Levy, J. A. (1973) *Carbohydr. Res.* 26, 393–400.
- [6] Tábora, E. (1976) Ph. D. Thesis, University of Buenos Aires.
- [7] Behrens, N. H., Carminatti, H., Staneloni, R. J., Leloir, L. F. and Cantarella, A. I. (1973) *Proc. Natl. Acad. Sci. USA* 70, 3390–3394.
- [8] Forsee, W. T. and Elbein, A. D. (1975) *J. Biol. Chem.* 250, 9283–9293.
- [9] Lucas, J. J., Waechter, C. J. and Lennarz, W. J. (1975) *J. Biol. Chem.* 250, 1992–2002.
- [10] Hsu, A. F., Baynes, J. W. and Heath, E. C. (1974) *Proc. Natl. Acad. Sci. USA* 71, 2391–2395.
- [11] Parodi, A. J., Behrens, N. H., Leloir, L. F. and Carminatti, H. (1972) *Proc. Natl. Acad. Sci. USA* 69, 3268–3272.
- [12] Sharma, C. B., Babczinski, P., Lehle, L. and Tanner, W. (1974) *Eur. J. Biochem.* 46, 35–41.
- [13] Lehle, L. and Tanner, W. (1975) *Biochim. Biophys. Acta* 399, 364–374.
- [14] Palamarczyk, G. and Chojnacki, T. (1973) *FEBS Lett.* 34, 201–203.
- [15] Behrens, N. H. and Leloir, L. F. (1970) *Proc. Natl. Acad. Sci. USA* 66, 153–159.
- [16] Lehle, L. and Tanner, W. (1974) *Biochim. Biophys. Acta* 350, 225–235.
- [17] Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature* 166, 444–445.
- [18] Behrens, N. H., Parodi, A. J. and Leloir, L. F. (1971) *Proc. Natl. Acad. Sci. USA* 68, 2857–2860.