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Note

Interference of guanosine (5′)-diphospho(1)-2-deoxy-α-D-*arabino*-hexose* with microsomal glycosylation-enzymes from *Aspergillus niger* van Tieghem

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Sugar analogs have been shown to interfere at various steps of the lipid-dependent glycosylation of proteins[1]. We have tested, *in vitro* with *Aspergillus niger* van Tieghem, which possesses a simple system of protein mannosylation *via* a unique D-mannosyl polyprenylphosphate intermediate[2], the influence of GDP-2-deGlc on the formation of the final glycosylated products as well as on the biosynthesis of dolichyl D-mannosyl phosphate.

When a three-fold molar excess of unlabeled GDP-2-deGlc over GDP-D-[^{14}C]mannose was added to the incubation medium, a strong inhibition of the

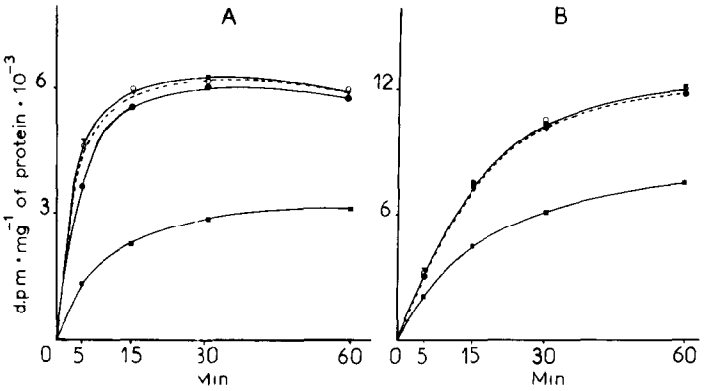

Fig. 1. Time course of transfer of D-[^{14}C]mannose from 20 nCi of GDP-D-[^{14}C]mannose (37 TBq/mol) to: endogenous polyprenyl phosphate (A) and proteins (B), in the presence of: 5.5μM GDP-D-Glc (▼——▼), 5.5μM GDP-2-deGlc (■——■), 5.5μM GDP (○——○), and 5.5μM UDP-2-deGlc (●——●); control (----). The incubation conditions are described in the Experimental section.

*Other systematic name: guanosine 5′-(2-deoxy-α-D-*arabino*-hexopyranosyl diphosphate). Trivial name: guanosine-diphospho-2-deoxyglucose (GDP-2-deGlc).

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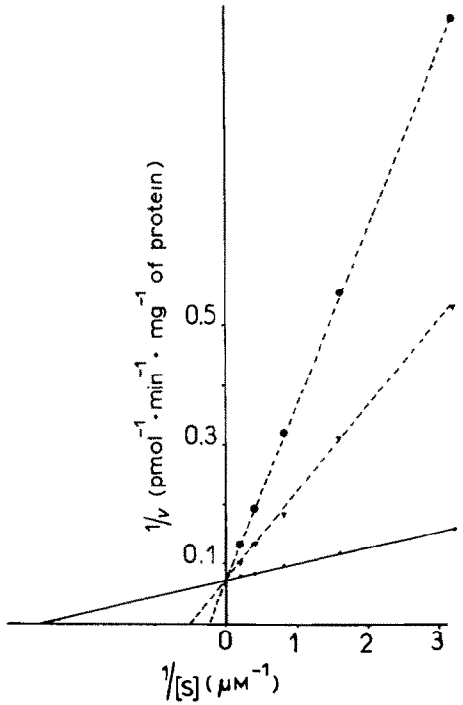

Fig. 2. Effect of GDP-2-deGlc on [^{14}C]mannose transfer from GDP-[^{14}C]mannose to endogenous polyprenyl phosphate. A Lineweaver-Burk plot (1/V *vs.* 1/S) was used: (·——·) control, (▼----▼) 5μM GDP-2-deGlc, and (●----●) 10μM GDP-2-deGlc.

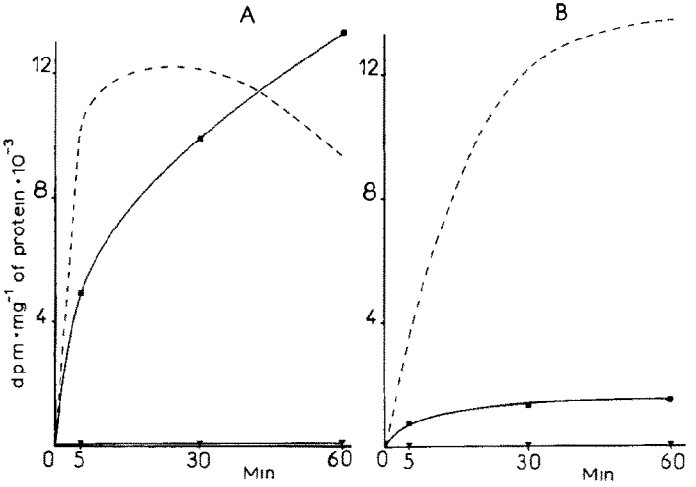

Fig. 3. Time course of transfer of D-[^{14}C]mannose, D-[^{14}C]glucose, and 2-deoxy-D-[^{14}C]*arabino*-hexose, from GDP-D-[^{14}C]mannose (----), GDP-D-[^{14}C]glucose (▼——▼), and GDP-2-de-[^{14}C]Glc (■——■) (0.74 kBq from 3.7 TBq/mol) to exogenous dolichyl phosphate (A) and proteins (B). Dol-P-loaded liposomes were prepared from pig liver as previously described[6].

polyprenyl [^{14}C]mannosyl phosphate synthesis was observed, whereas UDP-2-deGlc, GDP-Glc, and even GDP were inactive (Fig. 1A). The same level of inhibition was observed for D-mannosylation of the protein (the D-mannosyl residue is linked to serine or threonine[3]) (Fig. 1B).

Use of various ^{14}C-labeled diphospho-2-deoxyglucose nucleosides indicated that a radioactive lipid was formed only with GDP-2-deoxy[^{14}C]glucose. The kinetics of the synthesis of this lipid were very similar to that of mannosyl polyprenyl phosphate. Moreover, it showed the same chromatographic properties as mannosyl polyprenyl phosphate, and was slightly more acid-labile than the mannosyl derivative. No transfer of 2-deoxy-D-*arabino*-hexose to the proteins was observed (results not shown).

Study of [^{14}C]mannose transfer in presence of various concentrations of GDP-2-deGlc (Fig. 2) showed that the inhibition is truly competitive. The inhibitory constant determined from the double reciprocal plots has a very small value (Ki $\simeq 1\mu$M) characteristic of the great affinity of the enzyme for GDP-2-deGlc.

Previous results[4] having shown that dolichyl phosphate may be a rate-limiting factor, liposomes were used to obtain exogenous, Dol-P-enriched microsomes. Under these conditions, the specific activities of the GDP-mannose dolichyl phosphate-mannosyltransferase were 15 for GDP-D-mannose, 7 for GDP-2-deGlc (Fig. 3A), 1 for GDP-L-fucose[5], and 0.04 pmol·min^{-1}·mg^{-1} of protein for GDP-D-glucose[5].

These results indicate that the presence of the hydroxyl group at C-2 is very important for the transfer of the hexose residue to proteins; when the group is in an axial position, the transfer is possible, whereas an equatorial position blocks it. The proportion of 2-deoxy-D-*arabino*-hexose transferred from the polyprenyl phosphate derivative to proteins was very small (Fig. 3B), which seems to indicate that the enzyme responsible for the second phase of the transfer is even more dependent on the substitution at C-2 than the enzyme that forms the lipid intermediate from Dol-P and a nucleotide-diphosphohexose.

EXPERIMENTAL

The methods were the same as those previously reported[6].

Enzyme assay. — To an enzyme preparation from *Aspergillus niger* microsomes (100 μL containing 1 mg of proteins) in 50mM Tris·HCl buffer, pH 8.5, and 5mM $MgCl_2$ were added GDP-[^{14}C]mannose, or GDP- or UDP-2-de[^{14}C]Glc, or GDP-[^{14}C]glucose (0.74 kBq from 3.7 TBq/mol). The reaction was performed at 30° and stopped by addition of 2:1 (v/v) chloroform–methanol (2 mL). The ^{14}C-labeled lipids were extracted according to Folch *et al.*[7]. The insoluble material was layered on a glass-fiber filter (Whatman GF/C) and washed with the upper and lower theoretical phases[7].

ACKNOWLEDGMENTS

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