ACTION OF GLYCOSIDASES ON THE SACCHARIDE MOIETY OF THE GLUCOSE–CONTAINING DOLICHYL DIPHOSPHATE OLIGOSACCHARIDE

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1. Introduction

A key intermediate in protein glycosylation, is a dolichyl diphosphate oligosaccharide, which contains glucose, mannose and *N*-acetylglucosamine, and that was first detected as the product of glucose transfer from labelled dolichyl monophosphate-glucose [1]. This compound was referred to, first as glucosylated endogenous acceptor and more recently as dolichyl diphosphate-G-oligosaccharide [2]. Evidence has been accumulating which shows that 'in vivo' all the glucoses and part of the mannoses are removed from the G-oligosaccharide after its transfer to the protein [2–5].

This paper describes some experiments with a liver glucosidase which is probably involved in the process of removing the glucose 'in vivo' and which can be used to learn more about the structure of the G-oligosaccharide.

2. Materials and methods

Dolichyl diphosphate-G-oligosaccharide labelled in the glucose was prepared by incubation of liver microsomes with UDP-[^{14}C]glucose [6]. The same compound, but labelled in both glucose and mannose, was obtained by incubation of thyroid slices with [^{14}C]glucose [2,7]. Dolichyl diphosphate oligosaccharides labelled in the mannose and which are believed to be precursors of dolichyl diphosphate G-oligosaccharide were obtained by incubation of GDP-[^{14}C]-mannose with microsomes and a lipid extract of liver as acceptor [8]. All the compounds were then fractionated with chloroform–methanol–water mixtures and the oligosaccharides were obtained from them by mild acid hydrolysis [2].

Jack-bean α-mannosidase was purified up to the DEAE–Sephadex step [9]. Subcellular fractionation and the measurement of glycosidases with the appropriate *p*-nitrophenyl glycosides was carried out as in [10]. Marker enzymes were estimated according to [11].

Measurements of the action of glycosidases on the G-oligosaccharide were carried out by mixing about 5000 cpm substrate in 0.05 M triethanolamine acetate buffer (pH 6), 1% detergent Nonidet P-40 and about 0.2 mg protein in final vol. 50 μl. Samples were incubated for 10 min, 30 min and 120 min at 37°C; then 0.4 ml methanol, 0.15 ml water and 0.6 ml chloroform were added and the upper phase was concentrated, spotted on paper and chromatographed with butanol–pyridine–water (4:3:4). The radioactive zones were located with a scanner and then counted in a scintillator. The results were expressed as %-hexose liberated. The activity of the enzyme was calculated from the initial part of the time curves and corrected for protein content.

3. Results

Several commercial glucosidases were found to have hardly any activity on the G-oligosaccharides, i.e., Sigma yeast α-glucosidase, Sigma β-glucosidase from almonds, Miles mixed glycosidases from *Turbo cornutus* and Glaxo α-glucoamylase. It had been observed before that glucose is liberated when dolichyl

diphosphate-G-oligosaccharide was incubated with microsomes and Mn^{2+} ions [12]. Glucose production was attributed to hydrolysis of the oligosaccharide occuring after transfer to protein because conditions for optimal transfer led to increased liberation of glucose. The microsomal fraction was therefore selected for further studies. It was observed that glucose was liberated from the G-oligosaccharide, either free or combined with dolichyl diphosphate or with peptide. The rate of hydrolysis of G-oligosaccharide was increased about 4-fold by 1% Nonidet P-40. The pH optimum was 6. The following disaccharides, in order of decreasing rate, were hydrolyzed: maltose; kojibiose; nigerose. No activity was detected on cellobiose, αα-trehalose, isomaltose and laminaribiose.

The activity of the different subcellular fractions of liver is shown in table 1. The most active fraction in releasing glucose from the G-oligosaccharide was that of the endoplasmic reticulum, which also had the highest action on *p*-nitrophenyl α-D-glucoside. On the other hand, the most active fraction on the mannose-labelled oligosaccharide was the Golgi, which also had the highest activity on *p*-nitrophenyl α-D-mannoside, thus confirming the results in [13]. As expected, maximal activity of the marker enzymes, glucose 6-phosphatase and UDP galactose: *N*-acetylglucosamine galactosyl transferase, were found in the endoplasmic reticulum and Golgi, respectively.

The liver glucosidase was used in experiments carried out in order to learn more about the linkage of the glucose in the oligosaccharide. A sample of G-oligosaccharide labelled both in the glucose and in the mannose was treated with microsomal glucosidase and the product was chromatographed on paper with butanol–pyridine–water (6:4:3) as solvent. As shown in fig.1A, glucose, but no mannose, was released. The oligosaccharide remaining at the origin was eluted from the paper and rechromatographed with butanol–pyridine–water (4:3:4) which separates larger oligosaccharides. As shown in fig.1C the oligosaccharide

Table 1
Enzyme activities of different subcellular fractions of rat liver

	Total homogenate	Golgi fraction	Smooth endoplasmic fraction
Hydrolysis of G-oligosaccharide[a]	100[c]	50	250
Hydrolysis of [^{14}C]mannose-labelled oligosaccharide[a]	100[d]	600	100
α-Glucosidase[b]	1.6	2.0	5.4
α-Mannosidase[b]	2	40	14
Glucose 6-phosphatase[b]	120	83	200
UDP-galactose: *N*-acetylglucosamine galactosyl transferase[b]	0.1	2	0.1

[a] Rate of hydrolysis expressed as specific activity relative to the whole homogenate taken as 100
[b] Specific activities expressed in nmol. min^{-1} . mg^{-1}
[c] This value corresponds to 10% glucose liberated min^{-1} . mg^{-1}
[d] This value corresponds to 0.3% mannose liberated min^{-1}.mg^{-1}. This value cannot be compared with [c] because the specific radioactivity of the substrates was not known

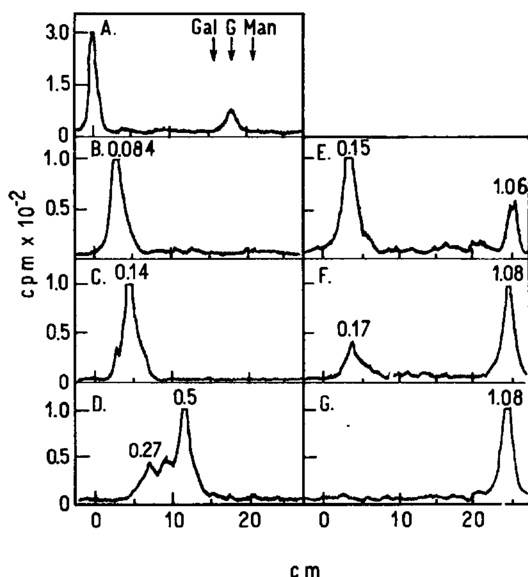

Fig.1. Effects of glycosidases on the oligosaccharides. (A) G-oligosaccharide ^{14}C-labelled in the glucose and mannose, treated with microsomal enzyme as in section 2, and chromatographed in butanol–pyridine–water (6:4:3). The following were chromatographed in a 4:3:4-composition solvent. (B) untreated sample; (C) slow substance of A eluted and rechromatographed; (D) untreated mannose-labelled oligosaccharides. The three cases on the right (E–G) correspond to B–D treated with 0.5 units Jack-bean α-mannosidase at 37°C for 20 h in total vol. 50 μl. The numbers correspond to R_{Glc}.

had an increased mobility as compared with the untreated sample (fig.1B). The change was estimated to correspond to a decrease of 2–3 hexose units as judged by the mobility of malto-oligosaccharides. When the samples were then treated with Jack-bean α-mannosidase, it was found that the oligosaccharide previously treated with the microsomal enzyme was nearly completely converted into mannose (fig.1F). Only a small peak remained, presumably due to the fact that glucosidase action had not been complete. As to the untreated G-oligosaccharide, it released less mannose (fig.1E) and its mobility was increased to an extent estimated to correspond to a decrease of about 3 hexose units. This change has been observed [4]. The mannose-labelled oligosaccharide behaved differently, since no radioactivity remained after the action of the Jack-bean mannosidase (fig.1C,D).

4. Discussion

The results showing that the liver α-glucosidase is found mainly in the endoplasmic reticulum fraction while α-mannosidase is more active in the Golgi fits well with the information we have on the G-oligosaccharide, according to which the processing has to start by removing the glucoses. This would occur in the endoplasmic reticulum, soon after or during polypeptide synthesis. The removal of the mannose would occur later when the glycoprotein reaches the Golgi.

The finding that the microsomal enzyme liberates only glucose from the G-oligosaccharide and makes most of the mannose residues susceptible to α-mannosidase can be explained by assigning the structure shown in fig.2 to the G-oligosaccharide. The α-anomeric structure of the glucose is deduced from the action of the microsomal enzyme, which does not split β-anomers. Previous experiments with yeast α-glucosidase led to the same conclusion but as large amounts of not-quite-pure enzyme and long incubation times were used, the conclusion was somewhat doubtful [14]. The glucoses are shown linked to one another because only ~50% radioactive glucose yields formic acid on periodate treatment [15]. The number of hexose residues is that suggested in [4] who measured the molecular size with appropriate standards. The mannoses are shown distributed in two chains, one consisting of three residues which is hydrolyzed directly by α-mannosidase, and another which is covered by the glucoses. The core pentasaccharide is the same as in the asparagine-linked oligosaccharides with two N-acetylglucosamines, as already known [15]. The structure proposed in fig.2 is only tentative but is compatible with most of the previous findings.

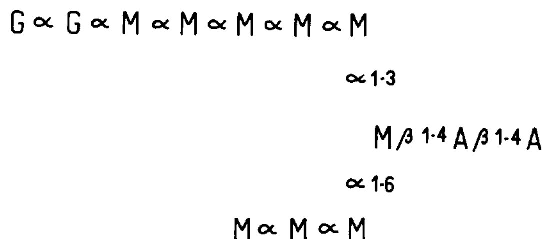

Fig.2. Tentative structure of the G-oligosaccharide. A stands for N-acetylglucosamine, M for mannose and G for glucose.

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