Lipid Intermediates in Glycosylation Reactions in Preneoplastic Nodules of the Liver *

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The protein-bound oligosaccharide chains of various membranes are considered to be important for normal structure and function. The composition of the sugar chain changes during carcinogenesis and several properties of the malignant cell can be related to this modified structure. The intracellular membranes of the rat liver contain almost exclusively *N*-glycosidically linked oligosaccharide chains, and the biosynthesis of these sugar chains required the participation of the lipid intermediate dolichol phosphate.

The role of dolichol in glycosylation reactions was investigated by using preneoplastic nodules of liver

induced by prolonged administration of 2-acetylaminofluorene to rats. The nodules were separated from the unchanged liver tissue and the homogenate or the microsomal fraction was used in the investigations as described earlier.¹ The dolichol content of the liver homogenate in rat was about 50 μg per g liver; this increased greatly in the nodules (Table 1). In the isolated microsomal fraction, the increase was even more dramatic than in the homogenate, which indicated that a large part, if not all, of the increase of the dolichol content in preneoplastic nodules was due to the increase in the microsomal fraction. The two major components in rat liver are dolichols with 18 and 19 isoprene residues while the 17, 20 and particularly the 21 residues large dolichols are present only in smaller amounts. This distribution pattern is unchanged in the case of nodules.

There are three major glycosyl transferases in liver microsomes which in some steps utilize lipid intermediates, and these were investigated here (Table 2). High incorporation of mannose, glucosamine, and glucose into endogenous dolichol phosphate of control microsomes takes place. All three sugars are transferred to endogenous protein acceptors which are present in the microsomes. Transfer of mannose and glucosamine, from the nucleotide activated form to dolichol and protein, decreases greatly when the analyses are performed on microsomes isolated from the nodules.

Dolichol phosphate may represent the rate limiting step in the glycosylation process, and

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Table 1. Distribution of different types of dolichols in control liver and in preneoplastic nodules. The dolichol content and distribution in homogenates and isolated microsomal fractions were measured by high pressure liquid chromatography. Eluates were monitored at 210 nm. In a convex gradient No. 4 (Waters) a solvent system, from the initial 2-propanol–methanol– H_2O (40:60:2) in pump system A to hexan–2-propanol (70:30) in pump system B was utilized.

Fraction	Amount μg per g liver	μg per mg protein	Control nodules microsomes % of total	
Control				
Total, homogenate	50.2			
Total, microsomes		0.26		
Nodules				
Total, homogenate	143			
Total, microsomes		1.45		
D17, microsomes			14	15
D18, "			41	40
D19, "			32	27
D20, "			10	12
D21, "			3	4

Table 2. Glycosylation of endogenous lipids and proteins in microsomes prepared from preneoplastic nodules. The incubation mixture contained 30 mM Tris-HCl, pH 7.8; 10 mM MnCl_2 ; 2.4 mM EDTA; 12.5 mM mercaptoethanol; 0.4% Triton X-100; 2 mM ATP and 30 kBq [^{14}C]GDP-mannose or [^{14}C]UDP-GlcNAc or [^{14}C]UDP-glucose and 2.5 mg microsomal protein in a final volume of 1 ml. After 15 min incubation at 30°C the dol-P, dol-PP-O and protein fraction were prepared as earlier.² O = oligosaccharide.

Substrate	Dol-P monosaccharide cpm per mg protein	Dol-PP-O	Protein
Control, microsomes			
GDP-Man	1804	120	560
UDP-GlcNAc	1175	90	868
UDP-Glc	918	44	564
Nodules, microsomes			
GDP-Man	1314	85	493
UDP-GlcNAc	765	68	491
UDP-Glc	910	38	498

Table 3. Glycosidase, kinase and phosphatase activities in microsomes from preneoplastic nodules. Glycosidases, kinases and dolichol monophosphatase were determined as earlier.^{2,4,5}

Exp.	Enzyme activity	Microsomes Control	Nodules
1	Glycosidases		
	α -Mannosidase ^a	1,6	1,5
	N-acetylglucosaminidase ^a	5,2	7,4
	Glucosidase ^a	2,7	2,5
2	Kinases		
	Dolichol-(D11)-P ^b	507	420
	Dolichol-(D20)-P ^b	150	80
3	Dolichol-monophosphatase		
	Dolichol-(D17-D21) ^c	3590	5700

^a $\mu\text{mol } p\text{-nitrophenyl/min mg protein}$. ^b Dolichol monophosphate formed (cpm/mg protein. 10 min). ^c Dolichol formed (cpm/mg prot. 10 min).

enzymes which directly regulate the amount of active intermediate are of utmost importance in glycoprotein metabolism.³ Furthermore, the results obtained in the studies of glycosyl transferases may be influenced by antagonistic enzymes since it has been established that glycosidases are not only lysosomal enzymes but are also present in the microsomal membranes. As shown in Table 3, α -mannosidase, glucosaminidase and glucosidase activities are present in control microsomes, and in the case of nodules the glucosaminidase activity is greatly increased. It is possible that the decrease of sugar transfer to lipid and protein

acceptors may be explained by limitation in the amount of dolichol phosphate, since the majority of the dolichol in the liver is present as free alcohol. The CTP-mediated kinase which is specific for dolichol phosphorylation plays a key role in the regulation of the active lipid intermediate.⁴ The kinase was measured with both dolichol-D11 and dolichol-D20 as a substrate and the activity in control microsomes was 3 times higher with the former. In the case of nodules the activity decreased with both substrates. Since dolichol phosphate concentration was low in the liver, the phosphatase present must have been

Table 4. Estimation of the amount of available dolichol phosphate for reaction with different sugars. Microsomes were incubated with 10 nmol radioactive sugar nucleotide (30 °C, 15 min) and the amount of lipid associated radioactive sugar in the chloroform extract was determined.⁶

Substrate	Sugar transfer to endogenous dolichol phosphate	
	Control	Nodules
	pmol/mg protein	
GDP-Man	16	12
UDP-GlcNAc	7	3
UDP-Glc	3	4

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efficient even under *in vivo* conditions. When the microsomes from nodules were tested, there was a significant increase in monophosphatase activity. These results which demonstrate decreased kinase and increased monophosphatase activities favor the idea that an actual decrease of dolichol phosphate amount occurs in preneoplastic nodules.

In order to estimate the maximal amount of dolichol phosphate which is functionally available as sugar acceptor from the nucleotide activated substrate, we employed an indirect approach⁶ (Table 4). Excess of substrate was used in incubation of microsomes, and, after 10 minutes incubation, the sugar transfer reached a plateau value because the amount of phosphorylated lipid intermediate was limited. In control microsomes, the capacity to accept mannose, glucosamine, and glucose was decreased in a proportion of about 4, 2 and 1, respectively. This indicates that different dolichol phosphates probably interact with the various sugars. In the case of nodules the microsomal dolichol phosphate capable for interaction with mannose and glucosamine exhibits a clear decrease.

The experiments described in this paper demonstrate that the amount of dolichol is greatly increased in the early phase of the neoplastic transformation. On the other hand, the results show that the amount of dolichol monophosphate probably decreases because of an increased phosphatase and a decreased phosphorylase activity. The final result is a decreased glycosylation of the endogenous protein.

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