The Effect of Chemical Carcinogens on the Dolichol Mediated Glycosylation of Rat Liver Microsomes *

CONNY EDLUND and GUSTAV DALLNER

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm and Department of Pathology at Huddinge Hospital, S-141 86 Huddinge, Sweden

Membranes of the malignant cell, particularly the plasma membrane, exhibit characteristic properties different from the normal cell. Chemical analysis of membranes prepared from experimental and human tumors has established the presence of glycoproteins which possess oligosaccharide chains with specific properties concerning structure and composition.[1] Glycosyl transferases are present in various cell membranes but the synthesis of the complete oligosaccharide chain requires the participation of enzymes in the endoplasmic reticulum. Dolichol phosphate is an obligatory intermediate in several steps of oligosaccharide synthesis and consequently its amount, composition and functional capacity is of great importance, as its role in rate limiting the biosynthetic process.[2] For this reason we performed a study of the initial effect of some chemical carcinogens on dolichol and dolichol mediated glycosylation in rat liver.

The amount and composition of dolichol in lipid extracts can be measured by high pressure liquid chromatography using a C18 reversed phase column.[3] Both liver homogenate and isolated microsomes prepared as described previously,[4] were analyzed for the effect of three chemical carcinogens and also phenobarbital, which is a known inducer of microsomal membranes and cytochrome P-450 (Table 1). Dolichol content in microsomes is changed significantly only after treatment of the rat with N-nitroso-diethylamine, which more than doubled the amount of lipid. On the other hand, treatment with the other substances decreased the amount of dolichol in the homogenate. The explanation for these differences between homogenate and microsomes is that the dolichol content of some intracellular membranes is considerably higher than in microsomes and, consequently, carcinogens exert obviously a differentiated effect on dolichol content at various locations. By comparison, dolichol content was also estimated in conditions where rapid synthesis takes place. In regenerating liver, the polyprene content in homogenate is significantly higher, while in microsomes it is somewhat lower than in the control. The first 3 days after birth, the amount of dolichol both in homogenate and microsomes is only 1/3 of that found in adult liver.

Microsomes were isolated from rat liver after various treatments and incubated with the 3 nucleotide sugars known to interact with dolichol monophosphate. This interaction is clearly influenced by the type of carcinogen administered (Table 2). Nitroso-diethylamine and methylcholanthrene decreased GlcNAc incorporation into the lipid intermediate, acetyl-aminofluorene and methylcholanthrene increased mannosylation of the lipid, while glucose transfer was increased by acetyl-aminofluorene and nitroso-diethylamine, and decreased by methylcholanthrene and phenobarbital. These experiments indicate that one of the factors regulating the biosynthesis of the oligosaccharide chain is the amount of the glycosylated lipid intermediate, which is the direct substrate of the glycosyl transferase acting for completion of the chain.

In addition to the glycosylated intermediate, the various glycosyl transferases also influence the structure of the carbohydrate chain on the protein (Table 3). The various treatments change only slightly the transfer of GlcNAc to the protein, but the transfer of mannose is decreased by nitroso-diethylamine and phenobarbital. Glycosylation of the protein on the other hand displays a uniform pattern since all the treatment employed lower the level of glucose transfer.

Rat liver homogenate contains a characteristic composition of dolichols dominated by C90 and C95 species. C85 and C100 forms are present in smaller amounts, while the C105 content is only around 4% (Table 4). This composition appears to be stable since none of the treatments cause any significant change in the distribution pattern of dolichols with various numbers of isoprenol residues.

The experiments described above demonstrate that the amount of dolichol, its active phosphorylated form and its capacity to function as a sugar acceptor are among the factors which determine the type of oligosaccharide chain which is synthesized and transferred to the protein, and participate in an *N*-glycosydic binding. Treatment of rats with chemical carcinogens changes the amount of dolichol in the liver but has no effect on the qualitative composition. Dolichol phosphate glycosylation is also modified as early as in the initial phase of the action of carcinogens the effect of which is a modified glycosylation of the endogenous protein acceptor.

*Communication at the Meeting of the Swedish Biochemical Society in Stockholm, 26–27th November, 1981.

0302-4369/82/070494-03$02.50
© 1982 Acta Chemica Scandinavica

Table 1. Dolichol contwnt in homogenate and microsomes from rat liver. Dolichol was isolated and quantitated by high pressure liquid chromatography. In exp . 1 rats were given intraperitoneally 2-acetyl-aminofluorene, 10 mg/100 g body weight; *N*-nitrosodiethylamine, 2 mg/100 g; 3-methylcholanthrene, 2 mg/100 g; and phenobarbital, 8 mg/100 g once a day during 5 days. Homogenate and microsomes were prepared on day 6. In exp. 2 partial hepatectomy was performed and the outgrown liver was removed 8 days later. In exp. 3 livers of newborn 6, 18 and 72 hours after birth were investigated. The values are the means ±S.E.M. of 6 experiments.

Exp.	Homogenate	Homogenate Dolichol μg/mg protein	Microsomes
1	Control	0.181±0.020	0.260±0.029
	2-Acetyl-aminofluorene	0.126±0.013	0.230±0.022
	N-Nitrosodiethylamine	0.379±0.028	0.605±0.052
	3-Methylcholanthrene	0.112±0.010	0.282±0.016
	Phenobarbital	0.078±0.005	0.230±0.019
2	Control	0.174±0.012	0.255±0.025
	Regenerating liver	0.271±0.027	0.219±0.029
3	6-Hour old	0.056±0.003	0.080±0.007
	18-Hour old	0.051±0.004	0.082±0.007
	72-Hour old	0.062±0.007	0.076±0.005

Table 2. Glycosylation of endogenous dolichol-P in liver microsomes of rats treated with chemical carcinogens. Microsomes were incubated with nucleotide-activated sugars as described earlier.[5] After incubation at 30 °C for 15 min the lipids were extracted by chloroform – methanol, 2:1 and after partition the radioactivity was determined in the chloroform fraction. The radioactive product was identified as dolichol-P by thin layer chromatography. The values represent the means of 4 experiments.

Treatment	GlcNAc	Mannose cpm per mg protein	Glucose
None	419	6 217	392
2-Acetyl-aminofluorene	427	12 306	512
N-Nitrosodiethylamine	340	3 873	461
3-Methylcholanthrene	349	17 850	305
Phenobarbital	447	5 262	336

Table 3. Glycosylation of endogenous protein in liver microsomes of rats treated with chemical carcinogens. Microsomes were incubated as described in Table 2 and after incubation the microsomes were extracted with chloroform – methanol, 2:1, and chloroform – methanol – H_2O, 1:1:0.3. The protein pellet was dissolved in 1 ml 2 % sodium dodecyl sulfate and radioactivity was measured by scintillation counting. The values are the means of 4 experiments.

Treatment	GlcNAc cpm per mg protein	Mannose	Glucose
None	169	239	422
2-Acetyl-aminofluorene	165	280	289
N-Nitrosodiethylamine	139	168	276
3-Methylcholanthrene	161	209	313
Phenobarbital	190	123	173

Table 4. Distribution of different types of dolichols in liver homogenates of rats treated with chemical carcinogens. The experimental conditions are described in Table 1. The individual dolichols were measured by high pressure liquid chromatography. The values represent the means of 3 experiments.

Treatment	Type of dolichol (% of total)				
	C 85	C 90	C 95	C 100	C 105
None	11.7	37.9	34.0	12.6	3.9
2-Acetyl-aminofluorene	11.7	41.5	32.7	10.6	3.5
N-Nitrosodiethylamine	12.1	35.7	33.0	14.3	4.9
3-Methylcholanthrene	8.3	39.3	34.3	13.2	4.9
Phenobarbital	9.2	38.1	32.7	13.5	6.5

Acknowledgement. This work was supported by the Swedish Medical Research Council and the Swedish Society of Medical Sciences.

1. Warren, L., Buck, C. A. and Tuszynski, G. P. *Biochim. Biophys. Acta 516* (1978) 97.
2. Dallner, G. and Hemming, F. W. In Lee, C. P., Schatz, G. and Dallner, G., Eds., *Mitochondria and Microsomes*, Addison-Wesley, Reading 1981, p. 655.
3. Tavares, I. A., Coolbear, T. and Hemming, F. W. *Arch. Biochem. Biophys. 207* (1981) 427.
4. Dallner, G. *Methods Enzymol. A 31* (1974) 191.
5. Bergman, A. and Dallner, G. *Biochim. Biophys. Acta 512* (1978) 123.

Received February 2, 1982.