Dolichol Distribution and Biosynthesis in Hepatocytes*

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Dolichol is present in hepatocytes in various amounts depending on the species.¹ In rat liver there is about 40 µg dolichol per gram wet weight of tissue but only a small part of the polyprenol is phosphorylated, that is, in an active form. This α -saturated polyprenol is an obligatory intermediate in several steps of glycoprotein synthesis and consequently the amount of lipid has considerable importance in regulating cell function.² Dolichol does not represent a designation for a single type of compound, rather a family of compounds with different numbers of isoprene residues. From liver to liver the number of isoprene residues ranges from 16 to 23. In order to obtain information about the mechanism of biosynthesis and the possible function of the different dolichols, experiments were performed by using isolated hepatocytes possessing the same, or nearly the same, properties as those in the intact liver.

Labeling of hepatocyte dolichol is a difficult problem mainly because of the lack of available precursors and of the metabolic pathway related to cholesterol. Since all the double bonds, with the

exception of the two at the Ω -end, are in *cis* configuration, it would be ideal to have mevalonate of the *S*-type which is, however, not available commercially. The first part of dolichol biosynthesis follows the route common to cholesterol, which is disadvantageous from the point of view of labeling, since the rate of cholesterol biosynthesis is at least a hundred times faster. Hepatocytes were isolated by perfusion first with an EGTA-containing medium followed by collagenase which yielded cells with over 90% viability. The cells were suspended in Krebs-Hensleit buffer and incubated for 5 h with [³H]-(*R,S*)-mevalonate having high specific activity (Table 1). In order to calculate specific radioactivity, we have measured the amount of dolichol by using high pressure liquid chromatography (HPLC).³ The dolichol content was about 40 µg in 1×10^8 cells and 70% consists of polyprenols with 18 and 19 isoprene residues. Smaller amounts were also present with 17, 20 and 21 residues. The procedure employed made it possible to label all of the dolichols that were isolated. The highest specific activity was obtained in the shortest dolichol with 16 residues, and the specific radioactivity decreased gradually reaching the lowest level in the longest dolichol. This finding would agree with a gradual addition of isopentenyl residues to the growing polyprenol chain occurring probably in the outer mitochondrial membrane.

In spite of the fact that during biosynthesis the dolichol product is in the pyrophosphate form, the large majority of the newly synthesized lipids are in dephosphorylated form and are stored either as free alcohols or esterified with a fatty acid. Consequently the amount of available active dolichol monophosphate is regulated by a CTP specific kinase.⁴ By using [³²P]CTP and an excess of exogenous dolichol(C55), we have studied the subcellular localization of the dolichol kinase by isolating subfractions from rat liver (Table 2). As opposed to Golgi membranes, outer and inner

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Table 1. Labeling of dolichol in hepatocytes with [³H]mevalonate. Hepatocytes at a number of 350×10^6 were incubated with 9.25 MBq [³H]-(*R,S*)-mevalonate for 5 h in 25 ml of Krebs-Hensleit buffer at 37 °C. After incubation the suspension was subjected to alkaline hydrolysis and dolichol was extracted with diethyl ether. The extract was placed on Al₂O₃ column and eluted with increasing concentration of diethyl ether in hexane. Dolichol measurements were made on HPLC.

Dolichol	Amount after purification		Radioactivity cpm/ 1×10^8 cells	cpm/nmol dolichol
	nmol 1×10^8 cells	% of total		
C80	0.25	2.0	509	2.027
C85	1.52	12.2	1.952	1.282
C90	4.73	38.2	2.444	517
C95	4.35	34.9	1.123	258
C100	1.58	12.7	352	222

Table 2. Phosphorylation of dolichol with [32 P]CTP. Subcellular fractions were incubated in a 500 μ l mixture containing 27 mM Tris-HCl, pH 7.0, 0.5 mM EDTA, 20 mM UTP, 5 mM β -mercaptoethanol, 30 mM CaCl_2 , 185 kBq [γ - 32 P]CTP, 40 μ M unlabeled CTP, 50 μ l dolichol (1 mg/ml) in 0.5% Triton X-100 and 1–7 mg protein. Incubation was at 30°C for 5 min. Dolichol phosphate was isolated by SiO_2 -column and thin layer chromatography.

Fraction	Dolichol mono-phosphate pmol/(mg protein)
Microsomes	11.0
Microsomes, trypsin-treated	0.4
Golgi membranes	1.2
Outer mitochondrial membranes	0.2
Inner mitochondrial membranes	0.3

mitochondrial membranes, the microsomes possessed a high capacity for dolichol phosphorylation. When microsomes were treated with trypsin before measurement of the kinase activity, dolichol phosphorylation was eliminated completely. This indicates that this enzyme is associated with the cytoplasmic surface of the microsomes.

The functional importance of different chain lengths in dolichols is not known and therefore attempts were made to enrich microsomal membranes in a well-defined polyprenol. Egg lecithin and dolichol were subjected to intensive

sonication which resulted in liposome formation. After a 90 min incubation about 5% of the dolichol entered into the hepatocyte and was present in all fractions (Table 3). The highest concentration appeared in microsomes and interestingly some of the lipids were distributed in the soluble cytoplasm. A portion of the dolichol, about 10%, was phosphorylated, indicating the functional importance of CTP-mediated microsomal dolichol kinase.

When the isolated microsomes, after preincubation with dolichol(C95), were incubated *in vitro* with nucleotide activated sugars, the newly incorporated lipid intermediate proved to be effective in accepting the various sugars. Glycosylation was most effective with mannose followed by glucose, with the lowest incorporation obtained with acetylglucosamine. In a moderate degree, glycosylation of endogenous proteins was also increased.

These experiments demonstrate that the hepatocyte system is suitable for investigation of dolichol biosynthesis by using labeled mevalonate as a precursor. One of the key enzymes in regulation of the amount of dolichol monophosphate is the CTP specific kinase, which is located exclusively on the outer surface of microsomal membranes. An effective way to study the specific role of dolichol in microsomes is to incubate the hepatocytes with the dolichol containing liposomes. This results in the uptake of dolichol, transport in the cytoplasm, and phosphorylation to an active intermediate.

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Table 3. Incubation of hepatocytes with liposomal [^3H]dolichol (C95). Hepatocytes at a number of 300×10^6 were incubated for 90 min in 25 ml of Krebs-Hensleit buffer at 37°C with egg lecithin liposomes containing 0.7 μ mol [^3H]dolichol (C95). After incubation fractionation was performed and lipids from the fractions were extracted with chloroform-methanol (2:1). Dolichol and dolichol monophosphate were isolated by chromatography on DEAE-Sephadex column.

Fraction	Dolichol pmol per mg protein	Dolichol mono-phosphate
300 g pellet	51.9	5.1
Mitochondria	46.0	5.9
10 000 g pellet	139.4	10.5
Microsomes	197.4	27.7
Supernatant	51.8	2.6

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