The Presence of Dolichol in Liver Supernatant *

E.L. APPELKVIST, T. CHOJNACKI and G. DALLNER

Department of Pathology at Huddinge Hospital, Karolinska Institutet, S-141 86 Stockholm and Department of Biochemistry, Arrheniuslaboratory, University of Stockholm, S-106 91 Stockholm, Sweden

All hepatocyte membranes contain dolichol in various amounts. The biosynthesis of this lipid occurs in the microsomes which means that a continuous transport of the substance must occur through the cytoplasm.[1] The situation is not unique since most phospholipids are also synthesized in the endoplasmic reticulum and transported by a specific lipid carrier to the other intracellular membranes.[2] In this study, we have analyzed the supernatant of the liver homogenate to find out whether or not dolichol is present at this particular location and if so whether or not it is associated with other macromolecules.

Lipid extraction of particle-free supernatant, produced by prolonged ultracentrifugation, demonstrated that dolichol is present in an amount of about 2 μg per g wet weight. Previously, it was found that Millipore filtration could be applied for particle size estimation when dealing with subcellular structures.[3] In such a test, rats were injected with [^{3}H]mevalonate into the portal vein; one hour later the liver was removed and the supernatant was prepared by prolonged centrifugation. The ensuing supernatant was divided into two parts: the upper 1 cm representing the top layer and the rest the middle layer. Fractions of these layers were passed through Millipore filters of various pore size and the percentage of dolichol that passed the filter was determined with high performance liquid chromatography (HPLC). The two fractions exhibited different behaviours (Table 1). In the top layer, most of the dolichol passed the 3μ filter but the passage was blocked at the 0.8μ filter size. The dolichol in the middle layer passed, however, to an appreciable extent even the smallest filter, 0.025μ. Obviously the dolichol in the top layer is associated with structures of a relatively large size in fact exceeding twice the size of the microsomes. The content of this fraction was analyzed in the electron microscope which revealed the presence of dense and homogeneous lipid droplets. The structures were surrounded by a membrane. It is possible that these large structures in the top layer do not primarily represent transport vesicles for dolichol but rather some kind of storage compartment.

Since dolichol is synthesized in the microsomes, its labeling is high already in the initial few minutes and the specific radioactivity declines rapidly in the first half an hour. It is also

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Table 1. Millipore filtration of the *in vivo* [^{3}H]mevalonate labeled supernatant. The supernatant fractions were applied to the filter and washed with 0.25 M sucrose. Numbers denote the precent of original dolichol concentration founded in the filtrate.

Filter μ	Dolichol, % of total in filtrate	
	Top layer	Middle layer
8	81	96
3	76	92
0.8	23	86
0.3	21	81
0.1	24	76
0.05	18	63
0.025	22	52

0302-4369/85 $2.50

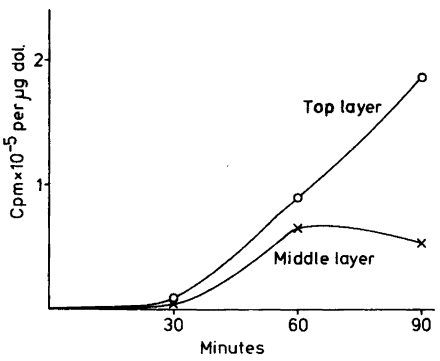

Fig. 1. Time course of *in vivo* labeling of liver supernatant dolichol with [^{3}H]mevalonate. Rats were injected with 19 MBq [^{3}H]mevalonate into the portal vein and the rats were decapitated at various intervals. The livers were homogenized and centrifuged for 3 h at 105 000*g*. After removal of the upper part (1 cm), or top layer, and the rest of the supernatant, called the middle layer, extraction was performed with chloroform-metanol 1:1 and dolichol was measured with HPLC as described previously.[4] The radioactivity in the total dolichol fraction was determined by liquid scintillation.

known, that, at the same time, the specific radioactivity of dolichol in the lysosomal compartment, which has the highest amount in hepatocyte, increases very slowly. When [^{3}H]mevalonate was injected into the portal vein, practically no dolichol labeling occurs in the two supernatant fractions within the first 30 min (Fig. 1). After this time, the radioactivity in the fractions increased rapidly in the second 30 min period and, in the middle layer, the plateau is reached. This pattern fits well into the behaviour of a transport substance mediating between microsomes and *e.g.* lysosomes. The specific dolichol activity of the top layer increases continuously during the investigated time (90 min) indicating that dolichol in this compartment differs functionally from that of the middle layer. The labeling pattern also excludes the possibility that dolichol is present in the supernatant as a result of contamination from membrane fragments or that it is a released and partially degraded membrane lipid.

Isolation of the fraction containing the polyprene was attained by filtration of the middle layer on Bio-Gel A (Fig. 2A). Almost all of the supernatant dolichol was recovered in the void volume, which indicates that dolichol is part of an aggregate or a lipid micelle. The polyprene contained in this fraction could also be precipitated with trichloroacetic acid.

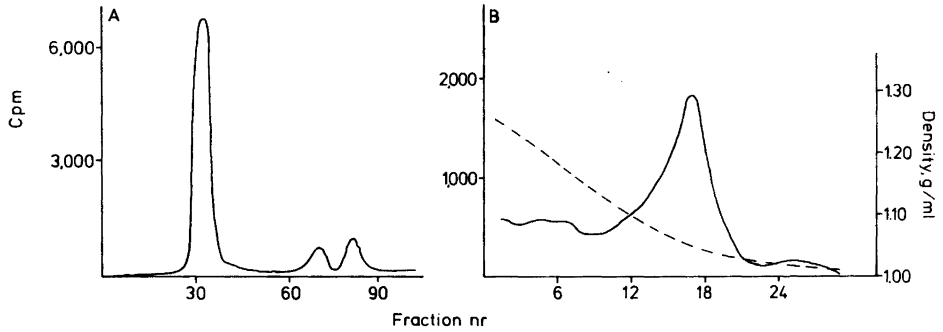

Fig. 2. Isolation of the dolichol containing fraction from the middle layer of the supernatant obtained from liver homogenate. A. The *in vivo* [^{3}H]mevalonate labeled supernatant was prepared 1 hr after the injection of the label. After homogenization and centrifugation, the middle layer was placed on a Bio-Gel A-1.5 m (1.5×70 cm) and was eluated with Tris-HCl, 50 mM, pH 7.8 with a flow rate of 0.2 ml/min. The individual fractions were analyzed for radioactivity in the isolated dolichol fraction. B. The peak in the void volume after Bio-Gel separation (10 ml) was mixed with 3.4 g KBr and placed on the bottom of a centrifuge tube. This mixture was overlayed with 0.9 %. NaCl and centrifuged in a vertical rotor at 200 000*g* for 150 min. After centrifugation, the density of the individual fractions was determined and the total radioactivity in the isolated dolichol was measured.

The dolichol recovery from the supernatant in the void volume necessitated an additional step of separation. The void volume after the Bio-Gel separation was mixed with KBr, overlayed with NaCl and centrifuged in a vertical rotor in order to develop a gradient. As it appears in Fig. 2B, the dolichol content is concentrated to one peak around the density of 1.05 g/ml. The use of a vertical rotor decreases considerably the time required for equilibrium.[5] Therefore the procedure is suitable for the preparation of large amounts of fractions containing dolichol, a necessary prerequisite for the characterization of a structure containing polyprene.

Chemical analysis of the peak fraction after gradient centrifugation also shows the presence of cholesterol and phospholipids. There are also proteins associated with this fraction, which, however, does not necessarily mean that specific protein carriers are components of the complex. Gel electrophoretic analysis gave a protein pattern, in some respects quite similar to the one found in the supernatant; therefore it is possible that the association is unspecific. The individual dolichol pattern is practically identical to the one that is found in the microsomes; the major peaks are those with 18 and 19 isoprene residues. The peak fraction after gradient centrifugation was also used for *in vitro* incorporation of dolichol with various subcellular membranes. It was found that transfer of the polyprene to isolated membranes actually took place, indicating the possibility that the polyprenes in the soluble phase participate in a transport process.

The results described here suggest that the hepatocyte cytoplasm contains dolichol in two forms, one as a large floating lipid vesicle and the other as a complex lipid structure containing phospholipids and cholesterol. Both lipid structures probably have transient appearances in the cytoplasm representing newly synthesized lipids necessary for the membrane biosynthetic process. The labeling pattern agrees with this type of function for the cytoplasmic non-membrane-bound dolichol.

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