Short Communication

Dolichol Biosynthesis in Rat Liver Peroxisomes*

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Dolichol is a polyisoprenoid substance present in all tissues of all higher animals, the highest level being found in human organs.[1] A portion of this lipid is esterified with fatty acids and another smaller portion is phosphorylated. Dolichyl-P is an obligatory intermediate in the biosynthesis of *N*-glycosidically linked oligosaccharide chains and thus plays a well established role in the biosynthesis of intra- and extracellular glycoproteins. The function of dolichol itself has not yet been established, but experimental data based on model membranes suggest its involvement in the regulation of membrane fluidity and stability, as well as implicating this compound as a mediator of membrane fusion processes.[2]

It is well established that the endoplasmic reticulum is the site of dolichol biosynthesis within the cell and that this lipid is subsequently transported to various organelles. Recently, it was demonstrated by immuno-electron microscopy that the rate-limiting enzyme of the mevalonate pathway, HMG–CoA reductase, is present not only on the endoplasmic reticulum, but also in peroxisomes.[3] Since peroxisomes contain enzymatic systems for β-oxidation of fatty acids, bile acid synthesis and the synthesis of plasmalogens, the possibility arose that this organelle also participates in dolichol biosynthesis. The present investigation sought to clarify this matter.

Results on particle composition are shown in Table 1. The amount of phospholipid in rat liver peroxisomes on a protein basis was found to be one-third of that in microsomes, mainly because of the large intraluminal protein content of peroxisomes. Peroxisomes were shown to have a low cholesterol content, but were enriched in dolichol relative to microsomes. HMG–CoA reductase activity was higher in peroxisomes than in microsomes and was higher in both of these fractions from fed rats than from starved animals. Catalase and NADPH-cytochrome *c* reductase, which are marker enzymes for peroxisomes and the endoplasmic reticulum, respectively, were also assayed and their distributions demonstrated that the fractions prepared here were cross-contaminated to only a very limited extent.

Polyprenol biosynthesis can be studied in microsomes with [^{3}H]-mevalonate as substrate in the presence of supernatant. An effective ATP-

Table 1. Composition of peroxisomes and microsomes.

	Peroxisomes	Microsomes
Phospholipid[a]	110	340
Cholesterol	2.2	21.4
Dolichol	0.51	0.15
HMG–CoA reductase[b]		
starved	38	15
non-starved	58	28
Catalase[c]	329	0.3
NADPH-cytochrome *c* reductase[d]	1.7	89

[a]μg/mg protein, [b]pmol/min/mg protein, [c]μmol H_2O_2/min/mg protein, [d]nmol cytochrome *c* reduced/min/mg protein.

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Table 2. Synthesis *in vitro* of dolichol from [^{3}H]-mevalonate.

Treatment *in vivo*	Peroxisomes	Microsomes
	% of control	
Control (fed)	100 (2202 dpm)	100 (3935 dpm)
Starved	77	21
Clofibrate	51	353
Di(2-ethylhexyl) phthalate	101	203
Cholesterol	91	121
Cholestyramine	119	48

generating system is necessary for effective biosynthesis. The major product is the α-unsaturated alcohol and subsequent saturation requires NADH, which is consumed in the enzymatic reduction of the α-terminal isoprene unit.

Table 2 shows results where dolichol is synthesized *in vitro* not only in microsomes but also by peroxisomes. Surprisingly, starvation decreased peroxisomal polyprenol synthesis only slightly, in contrast to the corresponding microsomal activity which was only 20 % of that obtained in fed rats. The rate of dolichol synthesis can be markedly changed by various treatments, which might be used to differentiate between the two sites of dolichol synthesis. For this reason, rats were furthermore treated with various inducers, and liver microsomes and peroxisomes were then prepared for use in *in vitro* studies.

Clofibrate, the well-known hypolipidemic agent and peroxisome proliferator, increased the rate of microsomal dolichol synthesis more than 3-fold relative to the fed control (Table 2), whereas the peroxisomal synthesis was decreased by 50 %. The plasticizer di(2-ethylhexyl) phthalate, which is also a peroxisome proliferator, increased the rate of polyprenol biosynthesis in microsomes but not in peroxisomes. A diet rich in cholesterol affects cholesterol biosynthesis markedly,[4] but did not influence dolichol synthesis in our experiments. Cholestyramine, which binds bile acids and is used for treatment of hyperlipidemic conditions, decreased microsomal polyprenol synthesis *in vitro,* but did not affect the peroxisomal rate. These experiments indicate that dolichol synthesis in microsomes and in peroxisomes is regulated by independent mechanisms.

Table 3. Synthesis *in vitro* of dolichol from [^{14}C]-isopentenyl-PP.

	Peroxisomes	Microsomes
	dpm/mg protein	
Control (fed)	926	185
Starved	893	177

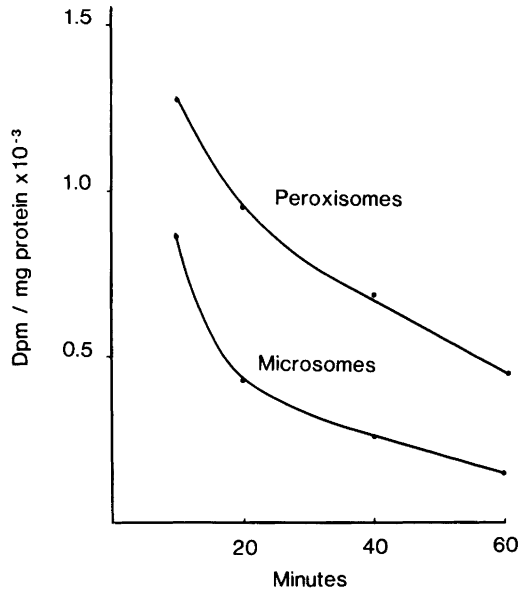

Fig. 1. Incorporation *in vivo* of [^{3}H]-mevalonate into peroxisomal and microsomal dolichol in rat liver.

The initial steps of the mevalonate pathway are common for cholesterol, ubiquinone and dolichol. The branching point is at the level of farnesyl-PP, where a series of condensation reactions with isopentenyl-PP occur, giving rise to α-unsaturated and α-saturated polyprenols and polyprenyl-P.[5] This portion of the biosynthetic pathway is mediated by membrane-bound microsomal enzymes which have not yet been studied in detail. Using [^{14}C]-isopentenyl-PP, it is possible to follow the terminal portion of polyprenol synthesis in the absence of the cytoplasmic enzymes.

Microsomes prepared from the livers of fed rats exhibited substantial polyprenol synthesis in the presence of [^{14}C]-isopentenyl-PP (Table 3). In

the same *in vitro* system, the synthetic rate in peroxisomes was 5 times higher, thereby indicating the presence of polyprenol synthesis in this organelle. Starvation did not influence the rate of isopentenyl incorporation in either of the organelles, a finding which is consistent with a regulation at preceding steps in the synthesis pathway, e.g. at the level of HMG–CoA reductase.

In order to study dolichol biosynthesis *in vivo,* [^{3}H]-mevalonate was injected into the intraportal vein of starved rats, peroxisomes and microsomes were isolated at various times thereafter, and the appearance of radioactivity in dolichol was monitored (Fig. 1). Ten min after injection, the incorporation of radioactivity into dolichol in both peroxisomes and microsomes was high, decreasing rapidly during the following 60 min. The extent of incorporation and the two somewhat different patterns of labelling in this system also support the idea of independent pathways of polyprenol synthesis in the endoplasmic reticulum and in the peroxisomes.

The experiments described here suggest that both dolichol and the enzymatic machinery for synthesizing this substance are present in peroxisomes. The rates of *in vitro* and *in vivo* peroxisomal dolichol synthesis and the different effects of various treatments exclude the possibility that the present results can be explained by microsomal contamination of the peroxisomal fraction. Because of the limited number of peroxisomes in hepatocytes, the contribution of this organelle to total hepatic polyprenol synthesis is probably limited. However, there are a large number of xenobiotics which are effective peroxisome proliferators. Some of these may also increase the rate of peroxisomal dolichol synthesis, which would alter the relative amounts of polyprenols synthesized by this organelle. In the terminal biosynthetic step, an α-saturation occurs concomitantly with the oxidation of NADH, while NAD is reduced during peroxisomal β-oxidation of fatty acids. In this way, dolichol synthesis may be stimulated by, and may be dependent on, other peroxisomal functions and, thereby, on quantitative changes in these functions.

Experimental

Groups of male Sprague-Dawley rats were treated in the following ways: starved for 20 h, maintained on a standard lab chow diet, maintained on diets containing clofibrate (0.6 %) or di (2-ethylhexyl) phthalate (2 %) for 7 d, or cholesterol (5 %) or cholestyramine (5 %) for 4 d. Isolation of peroxisomes and microsomes, as well as chemical and enzymatic measurements, were performed as described earlier.[6] To determine the rate of dolichol synthesis *in vitro,* isolated peroxisomes and microsomes were incubated with either 0.25 mCi of [^{3}H]-mevalonate (120 μmol/Ci) and the cytosolic fraction, or with 10 μCi of [^{14}C]-isopentenyl pyrophosphate (22 mmol/Ci) in 50 mM phosphate buffer, in the presence of 5 mM ATP, 10 mM phosphoenolpyruvate, 75 units of pyruvate kinase and 5 mM NADH. The incorporation of [^{3}H]-mevalonate into dolichols *in vivo* was studied by injecting 0.5 mCi of this precursor (13.8 Ci/mmol) into the portal vein of starved rats. The livers were then removed at various times and peroxisomes and microsomes prepared. The dolichol fractions were isolated using HPLC as described by Ekström *et al.*[7] and the total radioactivity in the peaks containing isoprenes 17–21 determined.

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References

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