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Biochimica et Biophysica Acta 1771 (2007) 506–513
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbalip

Defect in fatty acid esterification of dolichol in Niemann–Pick type C1 mouse livers in vivo

Mikael Turunen [a,1], Sophia Schedin-Weiss [b,*]

[a] Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden
[b] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

Received 2 October 2006; received in revised form 15 December 2006; accepted 2 January 2007
Available online 9 January 2007

Abstract

Fatty acid esterification of dolichol and cholesterol in Niemann–Pick type C1 mouse (Balb/c NIH npc1$^{-/-}$) livers was investigated in response to treatment with peroxisomal proliferators. These inducers have hypolipidemic properties and influence the mevalonate pathway and the intracellular transport of the final products of this biosynthetic route. Such inducers are consequently interesting to use in a disease model with defective intracellular transport of lipids. In wild-type mice, the levels of dolichol and cholesterol found as free alcohols were not changed to any great extent upon treatment with the peroxisomal inducers dehydroepiandrosterone, clofibrate and diethylhexylphtalate. In contrast, the amounts of dolichyl esters increased whereas cholesteryl esters decreased by the same treatments. The rate of enzymatic esterification of dolichol in isolated microsomes was accordingly elevated after 5- to 7-day treatments with the efficient peroxisomal proliferators DEHP and PFOA, while the corresponding esterification of cholesterol was decreased. Upon peroxisomal induction in npc1$^{-/-}$ mice, the enzymatic dolichol esterification in vitro increased whereas the low concentration of dolichyl esters remained unchanged. The results thus demonstrate that the induction of fatty acid esterification of dolichol in vivo is impaired in npc1$^{-/-}$ mouse liver. It is therefore proposed that the intracellular lipid transport defect in npc1$^{-/-}$ mouse liver disables either dolichol and/or the fatty acid from reaching the site of esterification in vivo. This proposal was strengthened by the finding that the amount of dolichol was decreased in an isolated Golgi fraction from npc1$^{-/-}$ mice.

Keywords: Dolichol; Dolichyl ester; Cholesteryl ester; Peroxisomal proliferation; Niemann–Pick type C disease; Lipid transport

1. Introduction

Dolichol is a long and hydrophobic isoprenoid lipid consisting of 16–23 isoprene units [1]. The first steps of its synthesis in animals and yeast occur via the mevalonate pathway, the isoprenoid biosynthetic pathway leading to the final products dolichol, cholesterol and other isoprenoids [2,3]. After the branch point, cis-prenyltransferase (CPT) catalyzes the chain elongation into long-chain polyprenol diphosphates that are subsequently dephosphorylated and reduced [4,5]. Dolichol exists in three forms: a free alcohol, phosphorylated or esterified with a fatty acid. The phosphorylated form of dolichol has a well-known function as a sugar carrier during N-glycosylation of proteins [6] and it has recently been suggested that the CPT activity controls the rate of N-glycosylation [5]. Polyisoprenols have also been suggested to be important in the organization of the glycosyltransferase complex during biosynthesis and translocation of N-glycosylated proteins by affecting membrane properties [7]. Except for the fusogenic and destabilizing properties on model membranes [7], the function(s) of the free and esterified forms are elusive. A recent report, however, demonstrated that free dolichol is the major lipid component of neuromelanin, a pigment present in high concentrations in dopaminergic neurons of human substantia

Abbreviations: NPC, Niemann–Pick type C disease; ADAT, acyl-coenzyme A dolichol acyltransferase; ACAT, acyl-coenzyme A cholesterol acyltransferase; dolichyl-P, dolichyl monophosphate; CPT, *cis*-prenyltransferase; DEHP, diethylhexylphtalate; PFOA, perfluorooctanoic acid; C:M, chloroform:methanol; C:M:W, chloroform:methanol:water

* Corresponding author.
E-mail address: Sophia.Schedin_Weiss@imbim.uu.se (S. Schedin-Weiss).
[1] Present address: Development DMPK and Bioanalysis, AstraZeneca R&D, Lund, Sweden.

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doi:10.1016/j.bbalip.2007.01.002

nigra [8], which implicates that dolichol has a role in the central nervous system.

The present knowledge about the intracellular transport of dolichol is scarce. An intracellular enzyme that catalyzes the esterification of dolichol, acyl-CoA dolichol acyltransferase (ADAT), is enriched in smooth II microsomes isolated from the liver, which is believed to be an early Golgi fraction [9,10]. Whereas dolichol is synthesized mainly in the ER [11], the highest levels of intracellular dolichol and dolichyl esters are found in Golgi vesicles and lysosomes [9,12]. In a previous study, it was suggested that the attachment of a fatty acid to dolichol is necessary for this lipid to be transported to lysosomes [9].

Metabolism of dolichol is altered in mice with Niemann–Pick type C disease (NPC) [13,14]. NPC is an autosomal, recessively inherited disease with progressive neurodegeneration and hepatosplenomegaly [15]. The most studied and prominent biochemical characteristic is the defective intracellular trafficking of LDL-derived cholesterol, leading to the accumulation of free cholesterol in late endosomes/lysosomes [16], but other lipids are also affected. The lipid storage pattern is complex and differs in various tissues. In humans, two genes, denoted NPC1 and NPC2, have been found to cause the NPC phenotype. NPC1 is responsible for ~95% of the cases. The NPC1 protein is a transmembrane protein found mainly in late endosomes, but also in lysosomes and Golgi [17]. The exact function of NPC1 is not known, except that it is involved in the transport of cholesterol and other lipids, either directly or indirectly [17]. Using a murine model of NPC1 disease ($npc1^{-/-}$) that displays the same genetic and biochemical defects as human patients, it has previously been shown that dolichol biosynthesis is decreased in livers from these mice in vivo and in vitro [14] and that the levels of dolichol and dolichyl phosphate are altered in different tissues of the diseased mice [13]. Additionally, certain peroxisomal enzyme activities are decreased, such as the peroxisomal β-oxidation of fatty acids [18]. Interestingly, treatment of affected mice with the potent peroxisomal proliferator, PFOA, was found to reduce cholesterol accumulation and normalize some down-regulated peroxisomal enzyme activities in $npc1^{-/-}$ livers [19]. Peroxisomal proliferators have hypolipidemic properties and are known to lower plasma triglycerides and cholesterol [20,21]. Additionally, they alter dolichol metabolism by affecting the activity of CTP [22].

In the present study, the extent of the esterification of dolichol was studied in mouse liver in response to treatment with different peroxisomal proliferators. Fatty acid esterification of dolichol increased in response to these drugs, in contrast to fatty acid esterification of cholesterol, which decreased. In $npc1^{-/-}$ mouse livers, induction of fatty acid esterification of dolichol in vitro, in an isolated Golgi/microsomal fraction, was similar to that of the wild-type mice. In contrast, induction of dolichyl ester formation in vivo, after clofibrate treatment, was abolished. The results thus indicate that the transport of dolichol and/or fatty acids to the site of dolichol esterification is impaired. This finding was strengthened by the discovery that the subcellular distribution of dolichol is altered in $npc1^{-/-}$ mouse livers, including lower levels of dolichol in the Golgi compartment.

2. Materials and methods

2.1. Materials

[^{14}C]Palmitoyl-CoA was purchased from Amersham. Lipoprotein lipase from *Pseudomonas* (76000 U/mg solid) was purchased from Sigma. Dolichol-23 was isolated from bovine pituitary glands according to the method of Radominska-Pyrek and coworkers [23].

2.2. Animals

The mutant BALB/c mouse strain $NPC1^{nih}$ was used for these experiments [24]. These mice contain a well-defined mutation in the npc1 gene. Homozygous mice carrying the mutant gene display a phenotype highly similar to the human disease and are referred to as $npc1^{-/-}$ in this work. Since the disease is recessively inherited and affected mice are not fertile, heterozygous mice ($npc1^{+/-}$) were used as breeders. The strain of BALB/c mice, from which the mutant mice were originally derived, was used as a control and is referred to as $npc1^{+/+}$ or wild-type in this work. $npc1^{-/-}$ mice were used 1–2 days after the development of visible symptoms, appearing at 5–7 weeks of age as staggering gate and, eventually, ataxia. Wild-type mice used as controls were age-matched in all experiments. Induction experiments were performed by supplying standard food containing either 0.5% DHEA, 0.5–1% clofibrate, 1% DEHP or 0.05% PFOA for 5–21 days. Since the biochemical defects as well as health problems accumulate with the age of $npc1^{-/-}$ mice, we used mice before the onset of visible symptoms in some experiments. In these cases, the zygosity of the mutant gene of the young mice, was determined by PCR. DNA was extracted with phenol after digesting the tip of the tail with proteinase K. The PCR reaction was performed using the primers GGTGCTGGACAGCCAAGTA and GATGGTCTGTTCTCCCATG, which flank the insertion event of the mutant gene. 30 cycles with 30 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C were used. The final extension step was 15 min.

2.3. Preparation of homogenates and subfractions

After decapitation of the animals, the livers were quickly removed and homogenized in 0.25 M cold sucrose by four up-and-down strokes at 440 rpm using a Potter–Elvehjelm homogenizer. Subfractions were isolated as detailed earlier [25]. Briefly, the pellet obtained by centrifugation at 2800×*g* was saved as a fraction enriched in heavy mitochondria/lysosomes, the pellet obtained at 25,300×*g* was saved as a fraction enriched in light mitochondria/lysosomes and finally, the pellet obtained by ultracentrifugation at 105,000×*g* was the microsomal fraction, also containing Golgi. The microsomal fraction was washed with buffer and recentrifuged at 105,000×*g* to obtain a purer microsomal fraction.

It is difficult to obtain subfractions from the livers of $npc1^{-/-}$ mice that are comparable to those from $npc1^{+/+}$ mice, since lipid-filled endosomes/lysosomes tend to contaminate most subfractions isolated from the livers of $npc1^{-/-}$ mice. In this study we tried density gradient centrifugation to isolate lysosomes, microsomes and Golgi from wild-type and $npc1^{-/-}$ mice. Homogenates were prepared as described above and centrifuged at 300×*g* to remove nuclei. The postnuclear supernatant was applied to 30% Percoll, essentially as described previously [26]. The microsomal/Golgi fraction was further applied to a sucrose gradient with 0.25, 0.8, 1.1 and 1.2 M sucrose layers, essentially as described before [27]. Lysosomal, Golgi and microsomal fractions were collected and marker enzyme activities were measured as described previously [9].

2.4. Enzyme assays

Microsomal ACAT activity was measured in 1 ml incubation medium containing 250 mM potassium phosphate, pH 6.4, 0.2% bovine serum albumin and 1 mg of microsomal protein. This mixture was preincubated at 37 °C for 10 min and the reaction was started by the addition of 1.5 μCi [^{14}C]palmitoyl-