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Review

Membranes and mammalian glycolipid transferring proteins

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ABSTRACT

Glycolipids are synthesized in and on various organelles throughout the cell. Their trafficking inside the cell is complex and involves both vesicular and protein-mediated machineries. Most important for the bulk lipid transport is the vesicular system, however, lipids moved by transfer proteins are also becoming more characterized. Here we review the latest advances in the glycolipid transfer protein (GLTP) and the phosphoinositol 4-phosphate adaptor protein-2 (FAPP2) field, from a membrane point of view.

Contents

Abbreviations: ARF, ADP-ribosylation factor; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; ER, endoplasmic reticulum; FAPP1/2, phosphoinositol 4-phosphate adaptor protein-1/2; FFAT, two phenylalanines (FF) in an acidic tract; GalCer, galactosylceramide; GCS, glucosylceramide synthase; GlcCer, glucosylceramide; GLTP, glycolipid transfer protein; GSL, glycosphingolipid; LacCer, lactosylceramide; OSBP, oxysterol-binding protein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PH, pleckstrin homology; PI, phosphatidylinositol; PI3P, phosphatidylinositol-3-phosphate; PI4P, phosphatidylinositol-4-phosphate; PM, plasma membrane; POPC, 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine; PS, phosphatidylserine; SM, sphingomyelin; START, steroidogenic acute regulatory protein (StAR)-related lipid transfer; TGN, trans-Golgi-network; VAP, vesicle-associated membrane protein-associated protein.

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1. Introduction

The hydrophobic nature of the lipids requires different transport and trafficking mechanisms compared to water soluble biomolecules. Lipids are synthesized on and in different organelles, and cells constantly need to adjust and respond to changes in the lipid requirements. To do this efficiently they need different transport machineries, connected to the synthesis and degradation pathways. Several mechanisms in the cells are used for lipid distribution. Most important for the bulk lipid transport is the vesicular system, however, lipid movement mediated by transfer proteins is also widely characterized. In addition, lipid transfer proteins have also often been given the role as sensors, responsible for coordinating the transport routes within the synthesis and degradation machineries. There are several glycolipid-binding proteins in mammals, proteins that specifically recognize glycolipids. The sphingolipid activator proteins (saposins) bind to different glycosphingolipids (GSL) and help in the degradation process of GSLs, with short oligosaccharide chains, in the lysosomes (Schulze et al., 2009). Mutations in the saposins can cause severe lysosomal storage disorders, like Gaucher disease (Schnabel et al., 1991). There is also evidence that non-specific lipid transfer proteins, isolated from beef liver, can recognize GSLs and transfer them between membranes (Bloj and Zilversmit, 1981). Another emerging group are the glycolipid transfer proteins. In this review we will focus mostly on glycolipid transfer protein (GLTP), but also on phosphoinositol 4-phosphate adaptor protein-2 (FAPP2), two proteins that belong to the GLTP superfamily.

2. Glycolipid transfer protein (GLTP)

GLTP is a small soluble, 24 kDa, protein that has been identified in many organisms. GLTP was first discovered in the cytosolic fraction from bovine spleen (Metz and Radin, 1980, 1982) and has since then been found in *e.g.* liver and brain from various mammalian sources (Abe et al., 1982; Yamada and Sasaki, 1982a, 1982b; Wong et al., 1984). In these studies GLTP was always purified from the cytosolic fraction and, consequently, the protein was postulated to be cytosolic. It has later been confirmed that GLTP indeed remains in the cytoplasm and not within other major cellular organelles (Tuuf and Mattjus, 2007). Homologs to mammalian GLTPs have furthermore been discovered in both plants (West et al., 2008) and yeast (Saupe et al., 1994). The expression levels of GLTP in bovine tissue have been analyzed (Lin et al., 2000). The results show that the cerebrum and kidney display the highest GLTP mRNA levels, which coincides with normally quite high amount of glycosphingolipids. GLTP can accelerate the transfer of glycolipids, both diacylglycerol- and sphingoid-based, between two lipid membranes. Substrates that can be used by GLTPs include glucosylceramide (GlcCer), lactosylceramide (LacCer), galactosylceramide (GalCer), sulfatide, GM_1, and GM_3 (Yamada et al., 1985; Brown et al., 1985). Furthermore, the initial sugar residue, linked to the ceramide or diacylglycerol backbone, needs to be in β-configuration for GLTP to recognize it as a substrate (Yamada et al., 1986). On the other hand, GLTP cannot transfer phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI), cholesterol and cholesterol oleate (Yamada et al., 1985). The activity of GLTP is sensitive to changes in the surrounding pH (West et al., 2006). Results show that GLTP has the highest transfer activity at pH 7.0, 66% transfer activity at pH 10 and only 11% activity at pH 4.0. This could give an important clue on GLTP subcellular localization and cellular activity, since GLTP will encounter different pH conditions within the cell.

The amino acid sequence of GLTPs from different mammals is highly conserved. The protein sequence of GLTP from pig brain was the first to be determined by Edman degradation (Abe, 1990). Later, it was demonstrated that GLTP contains 209 amino acids and that the sequence between porcine and bovine is 100% identical (Lin et al., 2000). Human GLTP has almost as high identity and shows 98% identity compared to porcine and bovine amino acid sequences (Li et al., 2004). Furthermore, the human GLTP amino acid sequence is 100% identical to the amino acid sequences of chimpanzee and macaque, 99% to the nonprimate mammal *Canis familaris* and 98% identical to the *Bos taurus* sequence (Zou et al., 2008). Two single-copy GLTP genes have been found in human cells, on chromosome 11 and 12. The transcriptionally active GLTP is on chromosome 12 and contains five exons and four introns, while the inactive pseudogene is located on chromosome 11 (Zou et al., 2008). The expression of GLTP mRNA is regulated by two transcription factors, Sp1 and Sp3. These factors bind to multiple sites in the GC-boxes localized to the promoter of GLTP (Zou et al., 2011). In the same study, the ability of different sphingolipids to enhance the GLTP transcription of GLTP was analyzed. Interestingly, only ceramide (but not for example GlcCer, GM_1 or sulfatide) could enhance the transcription levels of GLTP *via* the Sp1 and Sp3 transcription factors. The structure of both bovine and human GLTP has been determined, both in its apo-form and in complex with different glycolipids (Malinina et al., 2004; West et al., 2004; Airenne et al., 2006; Malinina et al., 2006). The GLTP structure contains eight α-helices, that are arranged in a two-layer fold, a structure that is completely novel for lipid transferring proteins. The carbohydrate group will bind to a recognition center on the surface of GLTP, through hydrophobic contacts and hydrogen bonding, and the hydrocarbon acyl chains will be accommodated into a hydrophobic pocket within the protein (Malinina et al., 2004). The binding of glycolipids to GLTP will be discussed extensively in Section 4.

The formation of GLTP dimers has been discussed in early publications. There are three cysteine residues in the GLTP sequence, two of them reside inside the protein and the third is on the surface of GLTP. Abe and coworker suggested that the two internal cysteines might form a intramolecular disulfide bond, while the cysteine on the outside could form a disulfide bond with another GLTP molecule (Abe and Sasaki, 1989a, 1989b). The disulfide bridge forming ability of GLTP was suggested to function as a means for regulating GLTP transfer activity, since it was demonstrated that the dimer had almost no transfer activity in contrast to GLTP with an intermolecular disulfide bridge, which had a high transfer activity. However, results from crystallization studies show no intra- or intermolecular disulfide bonds in the GLTP structure (Malinina et al., 2004; Airenne et al., 2006), but based on structural requirements it was suggested that it might be possible that an internal disulfide bridge would help in regulating GLTP activity (Airenne et al., 2006). It is still unknown whether disulfide bond-formation actually occurs *in vivo* as a regulatory mechanism for GLTP function. Recently a new transfer protein was discovered that moves ceramide-1-phosphate, and was named CPTP, ceramide-1-phosphate transfer protein (Simanshu et al., 2013). Structurally, CPTP has a very similar fold compared to the GLTP fold, however, CPTP associates with the trans-Golgi network (TGN), nucleus and the plasma membrane (PM). A CPTP-mediated ceramide-1-phosphate decrease in plasma membranes and increase in the Golgi complex stimulates $cPLA_2\alpha$ release of arachidonic acid, triggering pro-inflammatory eicosanoid generation.

3. Phosphoinositol 4-phosphate adaptor protein-2 (FAPP2)

There are two closely related homologs of four-phosphate adaptor proteins, type 1 and 2 (FAPP1 and FAPP2). FAPP1 was originally discovered in a study, when trying to identify novel proteins that could interact with various phosphatidylinositol-phosphates