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Specificity of the mammalian glycolipid transfer proteins

Peter Mattjus

Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

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ABSTRACT

Structurally the glycolipid transfer protein (GLTP) fold differs from other proteins that recognize glycolipids, such as non-specific lipid transfer proteins and lysosomal lipid degradation assisting proteins, even though they act on the same class of lipids. Proteins with glycan binding domains, such as lectins and pulmonary surfactant proteins share no structural similarity with the GLTP family either. Currently the unique GLTP-fold specific for binding glycosphingolipids is found only in the founding member GLTP and the phosphoinositol 4-phosphate adapter protein 2, FAPP2. FAPP2 was originally characterized as a member eight of the pleckstrin homology domain-containing family A (PLEKHA8). This review summarizes what is structurally required by the glycosphingolipids in order for them to be transported by the GLTPs.

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Raymond J. Metz when working on his thesis projects[1], under the supervision of Norman S. Radin, discovered[2] the 'CUP' protein in the late 70s (Metz and Radin, 1980). The project was a follow-up study stimulated by the earlier Dawson and Sweeley paper from 1970. Dawson and Sweeley (1970) reported glucosylceramide (GlcCer) exchange between red blood cells and plasma. The objective by Metz and Radin (1980) was to study if a protein could in fact have mediated the exchange of GlcCer seen by Dawson and Sweeley. They were indeed "dealing with a new protein", because they found that purified bovine spleen cytosol contained a protein that in vitro was accelerating a GlcCer exchange between rat erythrocytes and liposomes. Now we know the CUP protein (cerebroside uptake protein) as GLTP (glycolipid transfer protein). The acronym GLTP was later given by the Japanese team of Akira Abe, Keiko Yamada and Terukatsu Sasaki from the Sapporo Medical College. They extensively continued the study of GLTP in the late 80s (Sasaki, 1985, 1990). GLTP accelerates glycosphingolipid (GSL) intervesicular movement in vitro at least a thousand-fold compared to spontaneous transfer (Correa-Freire et al., 1982; Wong et al., 1984; Mattjus, 2009).

This review will summarize what is structurally required by the 'lipid ligands' in order for GLTP to see them as substrates, based on the different transfer assays and experimental setups published in the last 35 years. A handful of all the studies that today lay as a foundation for the physical and biochemical knowledge that we have about all the members in the glycolipid transfer protein superfamily[3], have ties to the Robert Bittman laboratory. Different structural modifications to sphingolipids have served as tools for us to better understand how the GLTPs work at membrane interfaces (Brown and Mattjus, 2007; Mattjus, 2009; Tuuf and Mattjus, 2013; Malinina et al., 2015). Lipid transfer events were indeed also familiar grounds for Bob. One of his earliest works were sterol and cholesterol transfer between different membranes (Rottem et al., 1978; Bittman et al., 1983).

1. Methods used to study lipid transfer protein activity

The in vitro transfer properties of GLTP have been studied using radionuclide and fluorescently labeled lipids. The early transfer assays usually employed natural membranes, that were sooner replaced by chain pure and structurally defined matrix lipids (Brown and Mattjus, 2007; Mattjus, 2009). We also have label-free approaches to study not only lipid transfer, but also lipid binding events (Locatelli-Hoops et al., 2006; Ohvo-Rekilä and Mattjus, 2011; D'Angelo et al., 2013).

1.1. Charged vesicle transfer assays and radiolabeled lipids

In this assay, donor vesicles are constructed so that they contain a negatively charged phospholipid (PL) while the acceptor vesicles are neutral. The charged donors can be separated from the neutral

E-mail address: pmattjus@abo.fi (P. Mattjus).

[1] The demonstration, purification, and characterization of a glucosylceramide transfer protein. Raymond J. Metz, thesis defense January 1, 1982, University of Michigan.

[2] Personal email communication, October 2005.

[3] http://supfam.org ID 110004.

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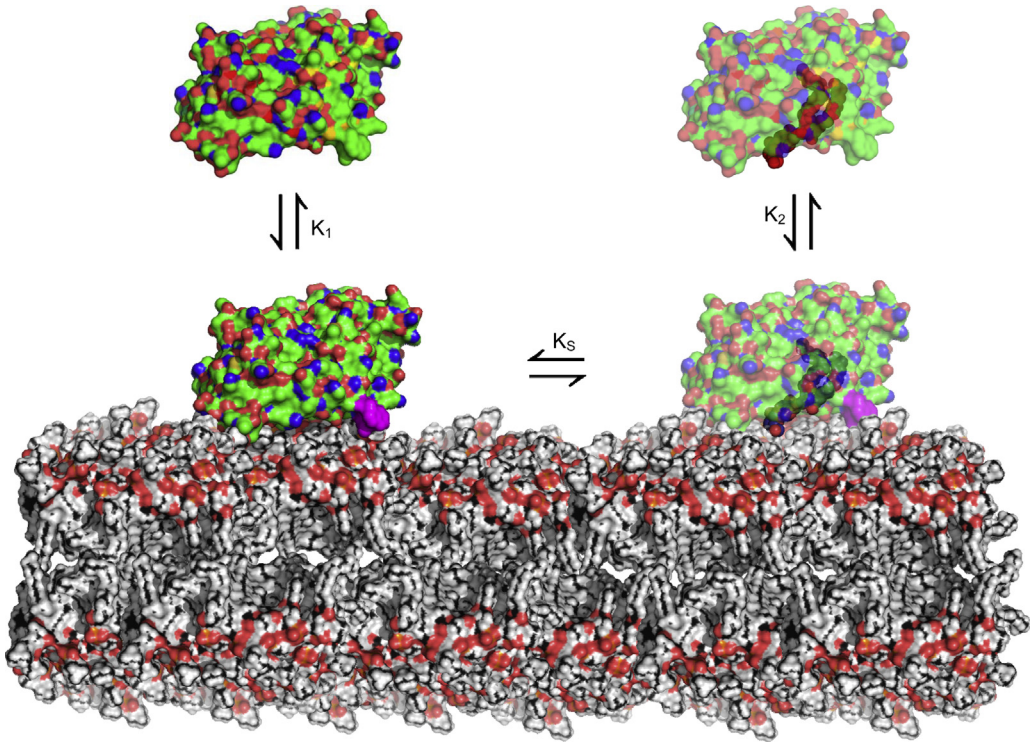

Fig. 1. Schematic illustration of the 'free transporter' mechanism of glycolipid transfer protein. GLTP partition onto the membrane surface in a non-specific, non-perturbing manner (K_1), regardless of ligand lipid presence or not (Rao et al., 2004). At the membrane leaflet aqueous interface GLTP interacts with the membrane. One of the parts of GLTP that is known to interact with the membrane is shown in purple. GLTP scans the membrane until a GLTP-lipid complex is formed, K_s. The rate-limiting step in the transfer is the formation of GLTP-lipid complex and the desorption from the membrane interface. In the next step conformational changes to the GLTP membrane interaction domains presumably cause the complex to be released into the aqueous environment, K_2. It is not known how the mechanism of the release of the glycolipid from GLTP into the acceptor membrane occurs. The GLTP (1WBE) and the membrane phospholipid molecules are rendered in the van der Waals volume (MacPyMol).

acceptor vesicles by ion exchange chromatography. Usually sonicated donor vesicles are used containing a charged PL like 5–10 mol % phosphatidic acid (PA), together with the radiolabeled glycolipid, usually ^{3}H-labeled and in a couple of mole percentage. To correct for spill over and vesicle fusion of donors into the acceptor vesicle pool, a trace of a non-exchangeable, usually a ^{14}C-labeled lipid, is incorporated into the donors. At desired time intervals, acceptors vesicles are separated from donor vesicles by elution over mini columns, and the eluate is analysed by scintillation counting. The amount of radioactivity in the different fractions corresponds to the transfer of glycolipids mediated by the transfer protein.

1.2. Brominated lipid transfer assays

Brominated acyl chains create heavier PLs that sediment faster compared to natural lipids, yet they form structurally equivalent bilayer lipid vesicles. This property has been used to separate donor and acceptor vesicles in transfer protein experiments (Wong et al., 1984; Brown et al., 1985). This assay allows the recovery of both the donor and acceptor vesicles with the use of density gradients and is both sensitive and reproducible. Tritiated glycolipids and a non-exchangeable ^{14}C-labeled lipid (such as cholesteryl oleate, or triolein) are incorporated into either non-brominated or brominated vesicles. The donor and acceptor vesicles are co-mixed and incubated with the transfer protein to be studied. The transfer reaction is terminated by fast centrifugation of the two vesicle populations over a step gradient in an Airfuge (Beckman). The separation needs to be done quickly and at high centrifugal forces, therefore the Airfuge has proven to be one of the only options available. The brominated vesicles and non-brominated vesicles are quantitatively well separated, and the transferred labeled glycolipids are finally measured by scintillation counting.

1.3. Fluorescence transfer assays

By far the most common lipid transfer assays use fluorescently labeled lipids. The setup usually involves a fluorophore pair, and are often called RET or FRET assays. In the donor vesicles one fluorophore, the energy donor, usually the transferred lipid is excited and its emission is quenched by the second fluorophore, the energy acceptor. The second fluorophore is a non-transferrable lipid that stays in the donor vesicles, at least well beyond the experimental time frame. Once the transfer protein starts to move the transferrable lipid to the acceptor vesicles, its emission is no longer quenched. The increase in emission as a function of time describes the transfer rate. Commonly used pairs are, BODIPY[4] and DiO-C16 (Nylund and Mattjus, 2005), anthrylvinyl and perylenoyl (Mattjus et al., 1999), NBD and rhodamine (Nichols and Pagano, 1983). Other fluorescence assay setups have also been used to analyse different transfer proteins. For instance, taking advantage of excimer/monomer fluorescence such as for pyrene (Brown et al., 1985; Somerharju et al., 1987) or self quenching of NBD (Nichols and Pagano, 1981) or parinaric acid (Somerharju et al., 1981).

1.4. Fluorescence competition assay

To analyse unlabeled lipids as potential substrates for transfer proteins a competition assay (Dansen et al., 1999) can be used

[4] BODIPY, dipyrromethene boron difluoride; DiO, 3,3-dioctadecyloxacarbocyanine perchlorate; NBD, 7-nitro-2-1,3-benzoxadiazol-4-yl.

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