Biochimica et Biophysica Acta 1831 (2013) 417–427

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

The glycolipid transfer protein (GLTP) domain of phosphoinositol 4-phosphate adaptor protein-2 (FAPP2): Structure drives preference for simple neutral glycosphingolipids

Ravi Kanth Kamlekar [a,1], Dhirendra K. Simanshu [b,1], Yong-guang Gao [a], Roopa Kenoth [a], Helen M. Pike [a], Franklyn G. Prendergast [c], Lucy Malinina [d], Julian G. Molotkovsky [e], Sergei Yu Venyaminov [c], Dinshaw J. Patel [b,*], Rhoderick E. Brown [a,**]

[a] The Hormel Institute, University of Minnesota, Austin, MN, USA
[b] Memorial Sloan-Kettering Cancer Center, New York, NY, USA
[c] Mayo Clinic College of Medicine, Rochester, MN, USA
[d] CIC BioGUNE, Parque Tecnológico de Vizcaya, Derio 48160, Spain
[e] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow 117997, Russia

ARTICLE INFO

Article history:
Received 4 September 2012
Received in revised form 23 October 2012
Accepted 29 October 2012
Available online 16 November 2012

Keywords:
Glycosphingolipid binding and transfer
GLTP superfamily
Membrane interaction
Tryptophan fluorescence
Near-UV and far-UV circular dichroism
Divergent evolution

ABSTRACT

Phosphoinositol 4-phosphate adaptor protein-2 (FAPP2) plays a key role in glycosphingolipid (GSL) production using its C-terminal domain to transport newly synthesized glucosylceramide away from the cytosol-facing glucosylceramide synthase in the cis-Golgi for further anabolic processing. Structural homology modeling against human glycolipid transfer protein (GLTP) predicts a GLTP-fold for FAPP2 C-terminal domain, but no experimental support exists to warrant inclusion in the GLTP superfamily. Here, the biophysical properties and glycolipid transfer specificity of FAPP2-C-terminal domain have been characterized and compared with other established GLTP-folds. Experimental evidence for a GLTP-fold includes: *i*) far-UV circular dichroism (CD) showing secondary structure with high alpha-helix content and a low thermally-induced unfolding transition (~41 °C); *ii*) near-UV-CD indicating only subtle tertiary conformational change before/after interaction with membranes containing/lacking glycolipid; *iii*) Red-shifted tryptophan (Trp) emission wavelength maximum (λ_{max} ~352 nm) for apo-FAPP2-C-terminal domain consistent with surface exposed intrinsic Trp residues; *iv*) 'signature' GLTP-fold Trp fluorescence response, i.e., intensity decrease (~30%) accompanied by strongly blue-shifted λ_{max} (~14 nm) upon interaction with membranes containing glycolipid, supporting direct involvement of Trp in glycolipid binding and enabling estimation of partitioning affinities. A structurally-based preference for other simple uncharged GSLs, in addition to glucosylceramide, makes human FAPP2-GLTP more similar to fungal HET-C2 than to plant AtGLTP1 (glucosylceramide-specific) or to broadly GSL-selective human GLTP. These findings along with the distinct mRNA exon/intron organizations originating from single-copy genes on separate human chromosomes suggest adaptive evolutionary divergence by these two GLTP-folds.

1. Introduction

The trans-Golgi network (TGN) functions as a major cell sorting complex, directing newly synthesized proteins and lipids to various subcellular destinations, receiving extracellular materials, and recycling molecules from endocytic compartments [1–3]. The exceptionally complex TGN sorting process involves mechanisms that regulate multiple divergent pathways to various acceptor compartments. Transport vesicle formation for various cargos involves attachment of distinct sets of docking and fusion factors (e.g. SNAREs) that target the correct acceptor compartment as well as the association of specific proteins that engage cytoskeletal tracks for vectorial trafficking. The maturation of transport vesicles destined for the plasma membrane often involves phosphoinositol 4-phosphate adaptor protein-2 (FAPP2) which uses its N-terminal pleckstrin homology domain to bind to phosphatidylinositol 4-phosphate in ADP-ribosylation-factor (ARF) dependent fashion and dock with Golgi membranes [4–8].

FAPP2 possesses a C-terminal domain with sequence homology to human glycolipid transfer protein (GLTP) [4,9,10]. This domain enables FAPP2 to transfer glucosylceramide (GlcCer) between cellular compartments. However, unlike GLTP (209 a.a.), FAPP2 (519 a.a.) contains an N-terminal pleckstrin homology (PH) domain that helps

* Corresponding author.
** Correspondence to: R.E. Brown, Univ. of Minnesota, Hormel Inst, 801 16th Avenue NE, Austin, MN 55912, USA. Tel.: +1 507 437 9625; fax: +1 507 437 9606.
E-mail addresses: pateld@mskcc.org (D.J. Patel), reb@umn.edu (R.E. Brown).
[1] Authors contributing equally to this study.

1388-1981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2012.10.010

to target the Golgi. D'Angelo et al. [11] have reported that complex glycosphingolipid (GSL) synthesis relies on FAPP2-mediated transfer of GlcCer from its synthetic site on the cytoplasmic face of the cis-Golgi to the trans-Golgi compartment. In contrast, Halter et al. [12] suggest that FAPP2 transfers GlcCer from the trans-Golgi to the endoplasmic reticulum before GlcCer returns to the Golgi for complex GSL synthesis. In any case, having two glycolipid transfer proteins, FAPP2 and GLTP, able to access cytosolic-facing membranes is important for maintaining cell viability. In vivo siRNA silencing that knocks down FAPP2 fails to block the arrival of newly synthesized GlcCer at the plasma membrane in the presence of the vesicle trafficking disruptor, brefeldin A, suggesting partial redundancy of GlcCer transfer by GLTP which is also able to deliver GlcCer to the plasma membrane in the presence of brefeldin A [12,13]. However, RNAi knockdown of both FAPP2 and GLTP results in cell death, suggesting a pivotal need for their functionality as glycolipid nonvesicular trafficking and/or sensor devices in cells. Downregulation of human FAPP2 gene (*PLEKHA8*; chromosome 7; locus 7p21-p11.2) by either target validation ribozymes or by FAPP2 siRNA also sensitizes cells to Fas/FasL-mediated apoptosis, emphasizing the need for normal FAPP2 expression to maintain cell viability and homeostasis [14].

Structural homology modeling of the FAPP2-C-terminal domain against the X-ray structure of human GLTP [15–17] predicts a GLTP-fold and topological conservation of key residues involved in glycolipid binding [10,11]. Yet, experimental data validating the FAPP2-C-terminal domain to be a GLTP-fold is lacking. Also, the exclusive focus on conserved core residues needed for sugar headgroup binding by the glycolipid recognition center without attention to residues that control GSL selectivity as well as transfer testing only of GlcCer and no other GSLs leave important issues unaddressed. Does the human FAPP2-C-terminal domain contain a GLTP-fold that has been evolutionarily adapted to transfer only GlcCer as reportedly occurs for plant GLTP1? Does transfer duplicity of human FAPP2 and human GLTP extend beyond GlcCer to include other glycolipids? In FAPP2, does the C-terminal domain structure or targeting by the N-terminal PH domain regulate glycolipid selectivity?

Herein, we have experimentally evaluated GLTP-fold formation by the FAPP2-C-terminal domain, determined its ability to transfer GSLs other than GlcCer, and identified local conformational changes expected to impact glycolipid binding specificity. Our experimental characterization includes assessment of intrinsic tryptophan fluorescence including 'signature' changes in intensity and emission wavelength maxima characteristic of GLTP-folds upon glycolipid binding as well as far-UV and near-UV CD spectroscopy to establish folding conformation, thermal stability, and changes induced by membrane interaction. Structural homology modeling against X-ray structures of human GLTP and fungal HET-C2 GLTP-fold [15,18] reveals local conformational changes expected to impact FAPP2 glycolipid selectivity. The results are discussed within the context of FAPP2 subcellular localization and evolutionary constraints imposed by the topology of GSL production and localization within cells.

2. Material and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), dipalmitoyl phosphatidic acid (DPPA), and all sphingolipids were obtained from Avanti Polar Lipids (Alabaster, Al) except for ganglioside GM_1 which was purified as described previously [19]. Radiolabeled galactosylceramide (GalCer; [galactose-6-^{3}H]; 20 Ci/mmol), glucosylceramide (GlcCer; [stearoyl-1-^{14}C]; 50 mCi/mmol), and sulfatide ([stearoyl-1-^{14}C]; 50 mCi/mmol) were purchased from American Radiolabeled Chemicals (Saint Louis, MO). Tritiated GM_1 and LacCer were prepared using galactose oxidase and tritiated borohydride and purified as described previously [19,20]. N-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-1-O-β-galactosylsphingosine (AV-GalCer) [21] and 1-acyl-2-[9-(3-perylenoyl)-nonanoyl]-*sn*-glycero-3-phosphocholine (Per-PC) were synthesized as detailed earlier [22]. Stock concentrations of phospholipids were quantitated by phosphate determination [23] and of glycolipids, by gravimetric analyses [24].

2.2. Methods

2.2.1. Cloning, expression and purification

FAPP2 encoded by human *PLEKHA8* was cloned from human brain cDNA (Clontech) by PCR using Herculase® II Fusion DNA polymerase (or Pfu Ultra High Fidelity DNA polymerase), forward primer 5′-GGA AGC GGA AAG ATG GAG GGG GTG CTG TAC AAG TGG-3′ and reverse primer 5′-GA GGA GAA GCC CGG TCA TAC CAC CTC ATC AGA TTC CAG-3′. Amplified ORF encoding FAPP2 (519 a.a.; identical with GenBank GI:119614336) served as template for cloning the C-terminal domain (212 a.a. = FAPP2-C212). Insertion at the BamHI and SalI restriction sites into pET-28 (kanamycin resistant; Novagen) modified with small ubiquitin-like modifier (SUMO) protein ORF enabled heterologous expression using transformed Rosetta cells grown in Luria–Bertani medium at 37 °C for 6 h, induced with 0.1 mM IPTG, and then grown 16–20 h at 20 °C. Purification from soluble lysate was achieved by Ni-NTA affinity chromatography. 6×His–SUMO-tag was removed from the N-terminus of FAPP2-C212 using a SUMO-specific protease, yielding a sequence identical to native protein. Final purification by FPLC SEC using a HiLoad 16/60 Superdex-75 prep grade column (Amersham) was verified by SDS-PAGE [20,25].

2.2.2. Protein concentration determination

A DU 640 spectrophotometer (Beckman) at a spectral bandwidth of 1.8 nm was used and the molar absorptivity was obtained by averaging the results from four calculation methods [26–29]. Spectra were corrected for turbidity by plotting the log dependence of the solution absorbance versus the log of the wavelength and extrapolating their linear dependence in the 340–440 nm to the 240–300 nm absorption range, using the DU-640 scatter correction routine. Extrapolated absorbance values were subtracted from the measured values, decreasing the apparent protein absorbance at 280 nm by ~15%.

2.2.3. Circular dichroism spectroscopy

CD spectra of FAPP2-C212 (~50 μM) were collected in 10 mM sodium phosphate (pH 7.4), and 30 mM NaCl at 10 °C while continuously purging with N_2 using a J810 spectropolarimeter (JASCO, Japan) equipped with a CTC-345 temperature-control system. CD spectra were recorded using ten accumulations, scanning each at 20 nm/min with a 2 s response time as detailed previously [18,30,31 & references therein] and presented in units of molar ellipticity per residue. Secondary structure was calculated from far-UV spectra using DichroWeb on-line analysis [32,33].

2.2.4. 3D-structural homology modeling

FAPP2-GLTP was analyzed using 3D-Jigsaw and I-TASSER comparative modeling algorithms, which build 3D-protein models based on homologs of known structure [34,35].

2.2.5. Fluorescence spectroscopy

Trp fluorescence was selectively monitored by excitation at 295 nm and measuring emission (310 to 420 nm) at 25 °C using a SPEX FluoroMax spectrofluorometer (Horiba Scientific, Edison NJ) as performed previously [18,25,30]. Band passes for excitation and emission were 5 nm. The cuvette was temperature-controlled to ±0.1 °C (Neslab RTE-111, Thermo Fisher, Waltham, MA). For membrane interaction studies, the emission signals of FAPP2-GLTP (1 μM) were measured before and after addition of various amounts of POPC vesicles lacking or containing 20 mole% glycolipid. Spectra were corrected by subtraction of buffer blanks. Inner filter effects were avoided by keeping the protein concentration at $OD_{295}<0.1$.