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The structure of complexes between phosphatidylethanolamine and glucosylceramide: A matrix for membrane rafts

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ABSTRACT

Interaction between membrane lipids creates lateral domains within which essential membrane processes like trans-membrane signaling, differentiation etc. take place. Attention has focused on liquid-ordered phases formed by sphingomyelin and cholesterol but formation of ordered domains on the cytoplasmic membrane surfaces has largely been neglected. Synchrotron X-ray powder diffraction methods were used to investigate the interaction between two components of the cytoplasmic leaflet of the plasma membrane, phosphatidylethanolamine and glucosylceramide. Multilamellar dispersions of binary mixtures of different molecular species of phosphatidylethanolamine and glucosylceramide were examined. Stoichiometric complexes are formed when the phosphatidylethanolamine has at least one unsaturated fatty acid. The stoichiometry of the complexes was 2.0 fluid phospholipids per glucosylceramide with C22/24 N-acyl chains and 1.8 with C-12 chains. Saturated molecular species of phosphatidylethanolamines were immiscible with glucosylceramide. The complexes formed with unsaturated phosphatidylethanolamines and glucosylceramide are stable above physiological temperatures. A putative role of these matrices in membrane rafts is considered.

1. Introduction

Our knowledge of the detailed structure of biological membranes is at a stage where it is possible to describe structure–function relationships in molecular terms. Thus the lipid bilayer does not simply act as a barrier to the diffusion of solutes but individual lipids are distributed asymmetrically across the bilayer and are organized into lateral domains by dynamic and specific interactions between the components. It is through these interactions that many membrane functions are executed. Thus dissipation of the gradient preserving phosphatidylserine on the cytoplasmic surface of apoptosing cells acts as an external "eat me" signal to macrophages [1]. Lateral organization of lipids, on the other hand, are said to function in transduction of membrane signals, membrane trafficking and differentiation and is associated with endo- and exocytotic processes [2–5]. There is now abundant evidence that lateral domains of lipids, referred to as membrane rafts are important elements in signaling processes by serving as platforms upon which lipid and protein components are assembled in a manner that brings appropriate components into functional association [6,7].

Lipid rafts are believed to be formed by interactions between cholesterol and sphingolipids which form a, so called, liquid-ordered phase [8,9]. This bilayer phase has properties intermediate between a gel and a fluid phase which segregates into discrete domains that are envisaged to act as dynamic organizing centers for raft-associated proteins [10]. Liquid-ordered domains of sphingomyelin and cholesterol have commanded most attention in this respect and both lipids are located on the outer leaflet of the plasma membrane and are prominent lipids present in isolated membrane rafts [11]. It has been shown that cholesterol–sphingomyelin complexes that form the liquid-ordered phase consist of an array of cholesterol molecules surrounded by 7 lipid hydrocarbon chains each of which is in contact with, on average, two cholesterol molecules [12]. The precise molecular arrangement of the complex is altered by the type and proportion of sphingolipids that comprise the complex. A case in point is the substitution of ceramide for sphingomyelin which has both structural and biochemical consequences pointing to the molecular mechanism of ceramide signaling in membranes [13–15].

Glycosphingolipids are also conspicuous components of membrane rafts. A characteristic feature of these glycosphingolipids is the long chain (C22–24) fatty acids N-linked to the long-chain sphingoid base. The homeostatic mechanisms regulating ceramide biosynthesis are the responsibility of Orm proteins which respond to appropriate levels of sphingolipids by acting as dynamic negative regulators of the key enzyme in synthesis of the long-chain base, serine palmitoyltransferase [16]. N-acylation of the long-chain base to form the ceramide is catalyzed by enzymes coded by a family of *Lass* genes (CerS1–6) each specific for substrate fatty acyl-CoA of particular chain length; CerS2 is largely responsible for producing the C22–24 molecular species [17]. Recent attention has been devoted to considering the significance of the length

Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; GlcCer, glucosylceramide; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering; L_{β}, lamellar gel phase; L_{α}, lamellar liquid-crystal phase

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of the N-acyl chain that comprises the ceramide moiety of sphingolipids in mediation of trans-membrane signaling processes [18] and their consequences in a variety of neurodegenerative diseases and conditions [19].

Less attention has been given to lipid domain structures in the cytoplasmic leaflet of cell membranes where glycosphingolipids are said to be less abundant and the dominant lipids are acidic phospholipids such as phosphatidylserine and phosphatidylethanolamine. The cytoplasmic leaflet contains glucosylceramide (GlcCer) which is synthesized at the cytoplasmic leaflet of Golgi membranes and translocated in the form of soluble protein complexes by glycolipid transfer protein [20] and phosphatidylinositol 4-phosphate adapter protein-2 [21] to the cytoplasmic leaflet of the endoplasmic reticulum and the plasma membrane [22,23]. About half of the GlcCer remains on the cytoplasmic leaflet and the rest is translocated to the cell surface [21]. The glucosylceramide is presumably targeted to the plasma membrane by an enrichment of the cytoplasmic leaflet with the signature glycophospholipid, PtdIns4-P, in a metabolic pool distinct from that of PtdIns (4,5)P-2 in this membrane [24]. Acidic phospholipids are also found abundantly in membrane rafts and these are confined to the cytoplasmic leaflet by the action P-4 ATPases catalyzing aminophospholipid translocation and which may also be involved with assembly of raft matrices [25].

In the present study I use synchrotron X-ray powder diffraction methods to show that interactions between phosphatidylethanolamines and glucosylceramide generate molecular complexes. The stoichiometry of the complexes is about 2 fluid phospholipid molecules per glucosylceramide and they are stable well above physiological temperatures. Detailed examination of different binary mixtures of the two lipids showed that only unsaturated, including, *trans* unsaturated, phospholipids form complexes; saturated molecular species are completely immiscible with the glycosphingolipid. The structure of these complexes is said to provide a relatively precise molecular scaffold for assembly of functional raft components on the cytoplasmic leaflet of the plasma membrane.

2. Materials and methods

2.1. Sample preparation

Phosphatidylethanolamines (PE) used in the study were 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE; 580 Da); 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE; 636 Da); 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE; 692 Da); 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE; 744 Da); 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE; 718 Da); 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE; 746 Da); 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphoethanolamine (OPPE; 718 Da); D-glucosyl-ß-1,1′ N-lauroyl-D-*erythro*-sphingosine (C-12 GlcCer; 644 Da) were purchased from Avanti Polar Lipid, Alabaster, and used without further purification. Natural ceramide β-D glucoside (glucocerebroside; GlcCer) extracted from the spleen of patients with Gaucher's disease was purchased from Matreya (Pleasant Gap, PA). GlcCer was >98% pure as judged by thin layer chromatography and the mean molecular weight is 812 g/mol. GlcCer obtained from this source consists mostly of a mixture of saturated, long acyl chains principally of lengths C-22 and C-24 [26]. GlcCer dissolved in chloroform/methanol (2/1, v/v) and phosphatidylethanolamines dissolved in chloroform were mixed in the desired proportions and dried under a stream of oxygen-free dry N_2. Any remaining traces of solvent were removed by storage under vacuum at 20 °C for 16 h. The dry lipids were hydrated with distilled water to give a dispersion of 25 wt.% lipid. The hydrated lipid samples were thermally cycled several times between −20 °C and 90 °C and vortex mixed to ensure homogeneous dispersion. The samples were stored at −20 °C and equilibrated for 5 h at 20 °C prior to transfer to the sample cells for X-ray diffraction examination.

2.2. Synchrotron X-ray diffraction methods

X-ray diffraction measurements were performed on beam-line 16.1 at the Daresbury Laboratory and BL40B2 at Spring8. The X-ray wave-length for the Daresbury experiments was 0.141 nm with a beam geometry of ~0.5×3 mm in a mica sandwich cell with a surface of 2×5 mm and a path length of 0.5 mm. Simultaneous SAXS and WAXS intensities were recorded so that a correlation could be established between the mesophase repeat spacings and the packing arrangement of acyl chains. The SAXS intensity was recorded using a quadrant detector giving a 1D scattering intensity profile of the powder pattern. The sample to SAXS detector distance was 1.5 m and calibration of d-spacings was performed using silver behenate (d = 5.838 nm). The WAXS intensity was recorded with an INEL (INstrumentation ELectronique, France) detector. Wide-angle X-ray scattering intensity profiles were calibrated using the diffraction peaks from high-density polyethylene [27]. The measurement cell was mounted on a programmable temperature stage (Linkam, Tadworth, UK) and the temperature was monitored by a thermocouple inserted directly into the lipid dispersion (Quad Service, Poissy, France). The set-up, calibration and facilities available on Station 16.1 are described comprehensively in the website; http://www.webarchive.org.uk/wayback/archive/20081018081841/, http://www.srs.ac.uk/srs/stations/station16.1.htm. Data collected at Spring8 was used to confirm phase assignments. An X-ray wave-length of 0.1 nm and a camera length of 400 mm were employed. Image plates were used to record the scattering intensity data. The sample environment, calibration and data processing were the same as that employed in the Daresbury experiments.

2.3. Analysis of X-ray diffraction data

The small-angle X-ray scattering-intensity profiles were analyzed using standard procedures [28]. Polarization and geometric corrections for line-width smearing were assessed by checking the symmetry of diffraction peaks in the present camera configuration using a sample of silver behenate. The orders of reflection could all be fitted by Gaussian + Lorentz symmetrical (Voigt) functions with fitting coefficients greater than $R^2 = 0.99$ (PeakFit 4.12, Systat software). Data reduction and analysis were performed using Originpro8 software (OriginLab Corp., Northampton, MA, USA). Deconvolution is consistent with the sample to detector distance used [29]. Peak fitting of Voigt functions to the first-order lamellar Bragg reflections and the wide-angle scattering bands was as described in the Supplementary Information of an earlier publication [30].

Spatial resolution of bilayer structures, taken to be Bragg spacing divided by the index of the highest detectible diffraction order, is known to be a measure of the widths of the distribution of constituents of the unit cell rather than the spatial separation of the distributions [31]. The parameter used to describe peak shape, amplitude/full width at half maximum amplitude, is therefore a measure of the relative order of the lipids constituting the bilayer repeat. Using the present peak fitting methods it is possible to distinguish positions of Bragg peaks in coexisting bilayer structures with a precision of at least 0.01 nm.

3. Results

3.1. Pure GlcCer

The structure and thermotropic phase transition behavior of an aqueous dispersion of GlcCer was examined first. Four orders of a lamellar reflection were observed in a sample equilibrated at 20 °C with a d-spacing of 5.0 nm and a scattering band in the WAXS region that can be resolved into a broad and a sharp peak of approximately