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Biochimica et Biophysica Acta 1768 (2007) 1628–1640
Biochimica et Biophysica Acta BBA
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Characterization of the conformational and orientational dynamics of ganglioside GM1 in a dipalmitoylphosphatidylcholine bilayer by molecular dynamics simulations

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Received 11 January 2007; received in revised form 10 February 2007; accepted 23 February 2007
Available online 7 March 2007

Abstract

The structure and dynamics of a single GM1 (Gal5-β1,3-GalNAc4-β1,4-(NeuAc3-α2,3)-Gal2-β1,4-Glc1-β1,1-Cer) embedded in a DPPC bilayer have been studied by MD simulations. Eleven simulations, each of 10 ns productive run, were performed with different initial conformations of GM1. Simulations of GM1-Os in water and of a DPPC bilayer were also performed to delineate the effects of the bilayer and GM1 on the conformational and orientational dynamics of each other. The conformation of the GM1 headgroup observed in the simulations is in agreement with those reported in literature; but the headgroup is restricted when embedded in the bilayer. NeuAc3 is the outermost saccharide towards the water phase. Glc1 and Gal2 prefer a parallel, and NeuAc3, GalNac4 and Gal5 prefer a perpendicular, orientation with respect to the bilayer normal. The overall characteristics of the bilayer are not affected by the presence of GM1; however, GM1 does influence the DPPC molecules in its immediate vicinity. The implications of these observations on the specific recognition and binding of GM1 embedded in a lipid bilayer by exogenous proteins as well as proteins embedded in lipids have been discussed.

Keywords: Glycolipids; Cell surface recognition; Lipid environment; DPPC bilayer; Local perturbation of bilayer; Conformational restriction

1. Introduction

Gangliosides are sialic acid-containing glycosphingolipids, occur ubiquitously in vertebrate cell membranes and are particularly abundant in the nervous system. Gangliosides are embedded in the outer layer of the plasma membrane by their hydrophobic ceramide moiety while the hydrophilic carbohydrate headgroup protrudes out of the membrane. Structural diversity and predominant localization on the outer layer of the plasma membrane enables gangliosides to participate in a number of cellular phenomena [1–9]. A large variety of proteins recognize and bind specific gangliosides present on the cell surface. In fact, the ability of gangliosides to act as cell surface receptors is exploited by certain bacteria for initial recognition and infection of the host cell [10,11].

The conformational behavior of ganglioside headgroups in lipid environment has been studied using NMR spectroscopic [12,13] and modeling [14,15] studies. Structural characteristics of gangliosides, such as diffusion constant, hydration, and packing, have also been studied when they are part of a lipid bilayer/micelle/vesicle [16–25]. However, atomic level details of packing and orientation of gangliosides with respect to the bilayer, the influence of surrounding lipid molecules on the ganglioside headgroup dynamics and the effect of ganglioside on the surrounding lipid molecules are not known. In view of these, MD simulations of a single GM1 molecule embedded in a hydrated DPPC bilayer have been performed in the present study. GM1-Os, the oligosaccharide moiety of GM1, and a hydrated

Abbreviations: Cer, Ceramide; DMPC, Dimyristoylphosphatidylcholine; DPPC, Dipalmitoylphosphatidylcholine; DPPE, Dipalmitoylphosphatidylethanolamine; EPC, Egg yolk phosphatidylcholine; FRAP, Fluorescence recovery after photobleaching; Gal, Galactose; GalNAc, 2-Deoxy-2-amino-N-acetylgalactosamine; Glc, Glucose; GM1, Gal-β1,3-GalNAc-β1,4-(NeuAc-α2,3)-Gal-β1,4-Glc-β1,1-Cer; GM1-Os, Oligosaccharide headgroup of GM1; MD, Molecular dynamics; NeuAc, N-Acetylneuraminic acid; POPC, Palmitoyloleoylphosphatidylcholine; SASA, Solvent accessible surface area

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doi:10.1016/j.bbamem.2007.02.020

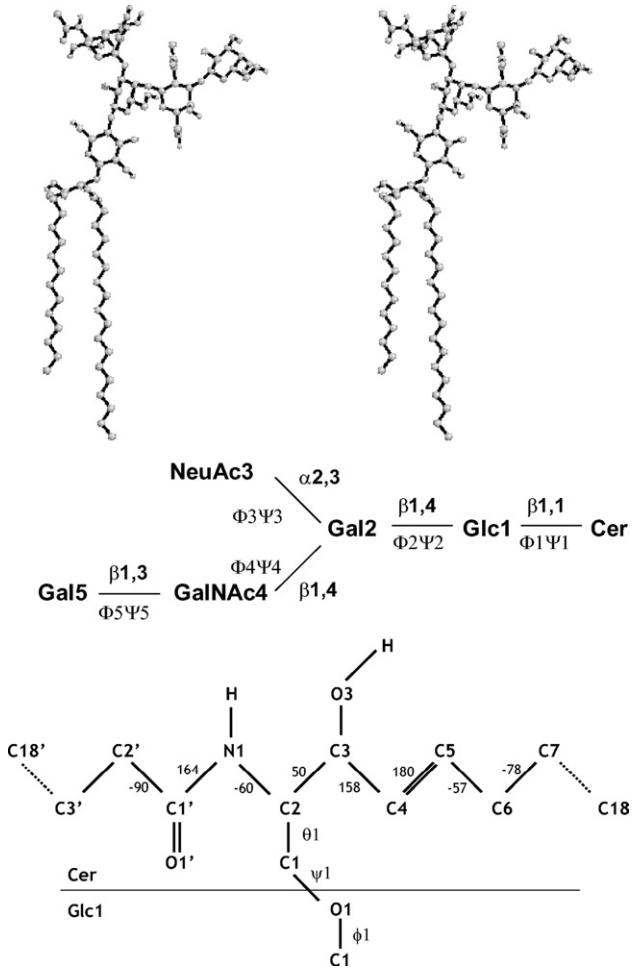

Fig. 1. Stereo view of the ball-and-stick representation of GM1 (top panel), schematic showing the residue and torsion angle numbering for the oligosaccharide headgroup of GM1 (middle panel) and atom nomenclature for ceramide (bottom panel). Initial conformations around the C–C bonds of the ceramide chain are trans except as indicated and these are from Ref. [28].

DPPC bilayer without GM1 have also been separately simulated to enable the delineation of the effect of lipid bilayer on the conformational and orientation dynamics of GM1 and vice-versa.

2. Methods

2.1. Conventions and definition

The residues of the oligosaccharide headgroup are numbered sequentially starting from the reducing end of the oligosaccharide (Fig. 1). The inter-saccharide linkages are given the same number as the non-reducing saccharide of that linkage. The inter-saccharide torsion angle ϕ is defined as H1–C1–O–CX′ except in NeuAc3-α2,3-Gal2 where it is taken as C1–C2–O–C3′; angle ψ is defined as C1–O–CX′–HX′. The conformation of the saccharide–ceramide linkage Glc1-β1,1-Cer is described by the angles ϕ1, ψ1, and θ1, which are defined as Glc1:H1-Glc1:C1-Glc1:O1-Cer:C1, Glc1:C1-Glc1:O1-Cer:C1-Cer:C2, and Glc1:O1-Cer:C1-Cer:C2-Cer:C3, respectively.

2.2. Initial conformations and bilayer configuration

The initial conformation of the various inter-saccharide linkages and the ceramide–saccharide linkage were chosen from literature [26,27] (Table 1). The conformations for the exocyclic hydroxyl groups were chosen randomly and those of the ceramide chain are as in Fig. 1 [28]. The exocyclic glycerol moiety of NeuAc3 was taken to be fully extended.

The DPPC bilayer configuration obtained at the end of the previously reported simulation ABI [29] was taken as the initial configuration for the present study. Water molecules were removed from this system and one DPPC molecule was replaced by a GM1 molecule. The latter was placed in such a way that the Z-coordinate of the Cer:O1′ atom (Fig. 1) is same as the average Z-coordinates of the carbonyl oxygen atoms of DPPC molecules in that monolayer. The system was energy minimized, enclosed in a box of size 4.9×6.1×11 nm and solvated. The water molecules present in the hydrophobic core of the lipid molecules were removed. One water molecule was replaced by a Na^+ ion using the *genion* module of GROMACS to neutralize the negative charge of the sialic acid moiety.

2.3. Simulation details

Eleven simulations that differed in the initial conformation of GM1 and/or the ceramide–saccharide linkage were carried out for the system consisting of 1 GM1 (126 atoms)+97 DPPC (50 atoms each)+1 Na^++water molecules (Table 1). After energy minimization, the system was simulated for 10 ps with the positions of GM1 and DPPC restrained for relaxing the solvent molecules. The system was further simulated to relax the GM1 molecule by restraining the positions of the DPPC molecules. This was followed by 1 ns of equilibration and 10 ns of productive run.

Eleven separate simulations were also run for GM1-Os, the pentasaccharide headgroup of GM1 molecule. The initial conformations are same as those used

Table 1
Initial conformation of GM1 and the number of water molecules in various simulations

Simulation [b]	Conformation [a]					Number of water molecules	
	ϕ1, ψ1, θ1	ϕ2, ψ2	ϕ3, ψ3	ϕ4, ψ4	ϕ5, ψ5	GM1-Os [c]	GM1 in DPPC
A	−60,93,60	165,6	−65,7	47,19	79,−22	2133	6664
B	−60,93,60	167,5	−60,−23	48,14	172,10	2135	6656
C	−60,93,60	54,7	−58,−24	54,9	170,14	2137	6655
D	−60,93,60	170,0	−170,4	151,11	−179,0	2133	6653
E	60,180,−60	60,0	180,−30	60,0	30,0	2129	6700
F	60,−60,180	30,0	−150,0	60,60	60,0	2132	6661
G	60,−60,180	60,0	180,0	30,−60	30,0	2130	6659
H	60,180,180	60,−60	150,0	60,0	60,60	2131	6682
I	−60,93,60	60,−60	−65,7	47,19	79,−22	2130	6655
J	60,−60,120	90,0	180,0	48,14	60,60	2130	6648
K	60,−60,180	30,0	60,0	60, 60	60,0	2130	6656

[a] The torsion angle nomenclature is as in Fig. 1.
[b] The suffix -Os is added for the simulations of GM1-Os.
[c] Simulations of GM1-Os.