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Molecular dynamics simulation of a phosphatidylglycerol membrane

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Abstract Although molecular dynamics simulations are an important tool for studying membrane systems, relatively few simulations have used anionic lipids. This paper reports the first simulation of a pure phosphatidylglycerol (PG) bilayer. The properties of this equilibrated palmitoyloleoylphosphatidylglycerol membrane agree with experimental observations of PG membranes and with previous simulations of monolayers and mixed bilayers containing PG lipids. These simulations also provide interesting insights into hydrogen bonding interactions in PG membranes. This equilibrated membrane will be a useful starting point for simulations of membrane proteins interacting with PG lipids.

Keywords: Membrane simulation; Phosphatidylglycerol; Palmitoyloleoylphosphatidylglycerol; Molecular dynamics

1. Introduction

Over the past decade, molecular dynamics (MD) simulations have been increasingly used to investigate the molecular-level interactions of lipid membranes and associated proteins [1–3]. However, most MD simulations use membranes made of zwitterionic lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Recently, a few simulations have been performed with membranes that include negatively charged lipids. For example, Pandit et al. and Mukhopadhyay et al. performed MD simulations of pure membranes of phosphatidylserine (PS), an anionic lipid that is commonly found in eukaryotic membranes [4,5]. Subsequent simulations were performed on mixed PC/PS membranes [6]. However, since many MD simulations involve bacterial membrane proteins or antimicrobial peptides that interact with bacterial membranes it is also critical to have good computer models of phosphatidylglycerol (PG), the anionic lipid most commonly found in prokaryotic membranes (Fig. 1A) [7]. A few papers have reported simulations of PG lipids, including simulations of PG monolayers [8] and a membrane containing a mixture of galactosylceramide and PG [9]. As well, Murzyn et al. have reported a MD simulation of a mixed PE/PG membrane, which represents a potentially useful model of bacterial membranes [10]. However, it is difficult to verify the validity of the force field parameters used for PG lipid in this situation since there is little experimental data on the structural characteristics and molecular interactions in mixed PE/PG membranes. Thus, a MD simulation of a pure PG membrane would be useful in order to validate the parameters used for PG lipids. Moreover, simulations of proteins with pure PG membranes can provide useful comparisons to experimental data on protein–lipid interactions obtained with pure PG lipid vesicles.

This paper describes the creation and equilibration of a pure palmitoyloleolyphosphatidylglycerol (POPG) lipid membrane in a 50 ns MD simulation. The properties of this equilibrated membrane are consistent with experimental observations on pure PG membranes, and it provides a useful starting point for future simulations of membrane proteins with PG membranes.

2. Materials and methods

An initial POPG molecule (Fig. 1A) was created in MacSpartan 02 (Wavefunction, Inc.) and subjected to 750 steps of steepest descents minimization using GROMACS. This lipid, and all others in the membrane, were in their negatively charged state based on the experimentally measured pK_a of PG [11] and the pH dependence of PG melting temperatures [12]. The minimized POPG was multiplied in an 8×8 grid to produce the first leaflet of the membrane, and the second leaflet was created by copying the first leaflet and rotating it appropriately. This bilayer was solvated with SPC water using the GROMACS genbox tool. All waters placed in the hydrophobic region of the membrane during this procedure were removed manually. Water molecules were replaced with 138 Na^+ and 10 Cl^- ions at the most favorable electrostatic positions to neutralize the overall system charge and provide an additional 100 mM salt concentration; after ions were added 5443 waters remained in the system. This system was minimized for 135 steps of steepest descents minimization, heated to 310 K over 20 ps and subjected to an additional 980 ps of MD. During this initial 1 ns simulation, reduced charges were used on the glycerol moiety of the headgroups. Five water molecules that quickly entered the hydrophobic region of the membrane during this initial simulation were then removed, and the simulation was continued for a 50 ns trajectory.

For comparison to the POPG membrane, a 10 ns simulation of a POPC membrane with 128 lipids and 2460 SPC waters was also performed using the same simulation parameters. This trajectory was started from the final frame of an equilibrated POPC simulation performed by Tieleman et al. and available at http://moose.bio.ucalgary.ca [13]. Since the POPC membrane had been previously equilibrated, albeit under somewhat different simulation parameters, its properties converged very quickly. Values reported for the POPC system are averaged over the last 5 ns of the trajectory.

All MD simulations and analyses were performed with GROMACS v3.2 [14]. Simulations utilized the NPT ensemble with anisotropic pressure coupling ($\tau_p = 1.0$ ps) to 1 bar in each direction and temperature coupling ($\tau_t = 0.1$ ps) of lipid, water, Na^+ and Cl^- separately to 310 K. Berendsen coupling protocols were used for both pressure

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Abbreviations: MD, molecular dynamics; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; POPG, palmitoyloleoylphosphatidylglycerol; RDF, radial distribution function

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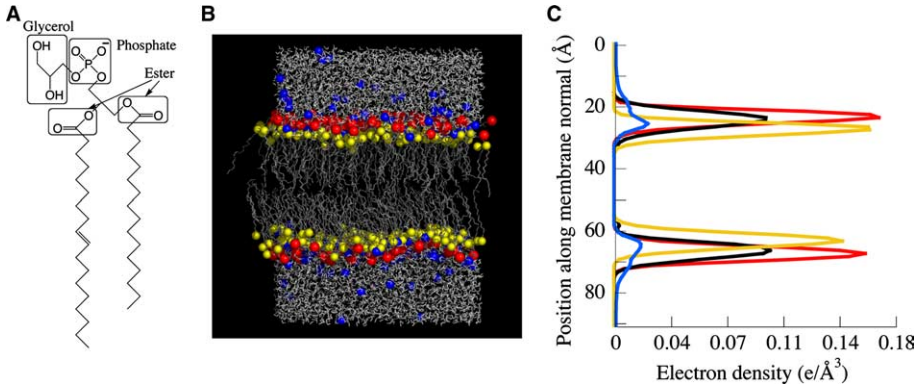

Fig. 1. (A) Molecular structure of POPG, with the groups used for analyses highlighted. (B) 50 ns frame of the POPG simulation. Lipid and water are shown as gray and white line structures, respectively. Lipid phosphorous atoms, the oxygen atoms of lipid ester groups, and sodium ions are shown in red, yellow, and blue spacefilling, respectively. Chloride ions are omitted from the figure for clarity. (C) Electron density profiles of the lipid phosphorous (red), glycerol (black), and ester (yellow) groups and sodium cations (blue) along the membrane normal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and temperature [15]. Long-range electrostatics were computed using the particle mesh Ewald (PME) method [16] as recommended for membrane simulations, especially those involving charged lipids [17,18]. Lennard-Jones energies were cutoff at 1.0 nm. Bond lengths were constrained with LINCS [19] and water geometries were constrained with SETTLE [20]. Parameters for the POPC membrane were the same as those used previously [13], with lipid tail parameters taken from Berger et al. [21]. POPG parameters were the same as those of POPC for identical portions of the molecules with glycerol charges based on the standard GROMACS force field and other glycerol parameters assigned by the PRODRG server [22]. POPG parameters and coordinates for the equilibrated POPG membrane can be downloaded from http://www.wellesley.edu/Chemistry/Don/home.html.

Analyses were primarily performed using tools available in the GROMACS suite. Errors reported for averages are standard deviations. Hydrogen bonds were determined geometrically with a donor–acceptor distance cutoff of 3.5 Å and an angle cutoff of 30°, unless otherwise noted. Deuterium order parameters (S_{CD}) were calculated as described elsewhere [4]. Molecular graphics were rendered using Pymol (http://www.pymol.org).

3. Results and discussion

3.1. Equilibration and structural properties of the POPG membrane

The POPG membrane clearly equilibrates during the 50 ns simulation based on both the energy of the system and the convergence of lipid membrane structural properties (Fig. 2). In general, these and other properties reached equilibration during the first half of the trajectory, and average properties calculated over the last 25 and 10 ns were essentially identical. Throughout the rest of this paper all POPG properties reported are averaged over the final 10 ns of the trajectory. The 50 ns frame of the POPG simulation is shown in Fig. 1B. In general, the POPG phosphate and glycerol groups were at a similar depth in the membrane, with the ester groups residing somewhat deeper in the membrane (Fig. 1B and C). This is quite similar to PS membrane simulations, in which the terminal carboxylate group resides at essentially the same depth as the phosphate groups [4,5].

Differences in the area per lipid and thickness of the equilibrated POPG and POPC membranes were consistent with past experimental measurements. The average area per lipid in the equilibrated POPG membrane was lower than that in POPC (56.1 ± 0.7 Å^2 versus 68.4 ± 0.5 Å^2). Conversely, the POPG membrane was thicker than the POPC membrane, as measured by the distance between phosphorous atoms in the two membrane leaflets (42.7 ± 0.6 Å versus 36.8 ± 0.6 Å). Although there are no direct experimental measurements of POPG and POPC membrane structures in the literature, our simulations reflect general trends between PG and PC membranes. For example, gel phase dipalmitoylphosphatidylglycerol (DPPG) membranes had a smaller area per lipid and were thicker than dipalmitoylphosphatidylcholine (DPPC) membranes; the same is true for membranes made of egg PG and PC lipids [23–26]. The area per lipid in the POPG simulation was significantly smaller than the area per POPG lipid in a mixed POPE/POPG membrane simulation (62.8 Å^2) [10]. This difference is not particularly surprising, since the mixed membrane is only 25% POPG and the large amount of zwitterionic POPE present likely affects POPG properties. As well, the mixed lipid environment also may alter the area per POPE in that simulation, which is rather similar to the area per lipid in pure POPC membrane simulations using the same force field. Experimental measurements of pure lipid membranes show that the area per PE lipid is ≈10% smaller than that of PC lipids [27].

POPG lipid tails were more ordered than those of POPC in these simulations, as shown by their deuterium order parameters (S_{CD}) (Fig. 3A and B). Previous simulations of PG monolayers and mixed PE/PG membranes also showed that PG lipid tails appear to be more ordered than PC lipid tails [8,10]. A similar ordering of anionic lipid tails relative to the tails of zwitterionic lipids was also observed in simulations of PS membranes [4,5].

On the average, the vector connecting P and O in PG headgroups was slightly pointed into the membrane (average angle ≈102°), although a broad distribution of angles was observed in the equilibrated membrane (Fig. 3C). In contrast, the P–N vector in PS simulations tended to point into the water [4,5]. This difference likely arises since the PS amine group is positively charged, whereas the PG glycerol is neutral.

3.2. Hydrogen bonding of POPG

In this simulation, the POPG phosphate groups had an interesting balance between interactions with water and with the glycerol moieties of adjacent lipids. In particular, the radial distribution function (RDF) for phosphate groups shows that while glycerol groups have the closest interactions with phosphates, hydration shells also form around the phosphate

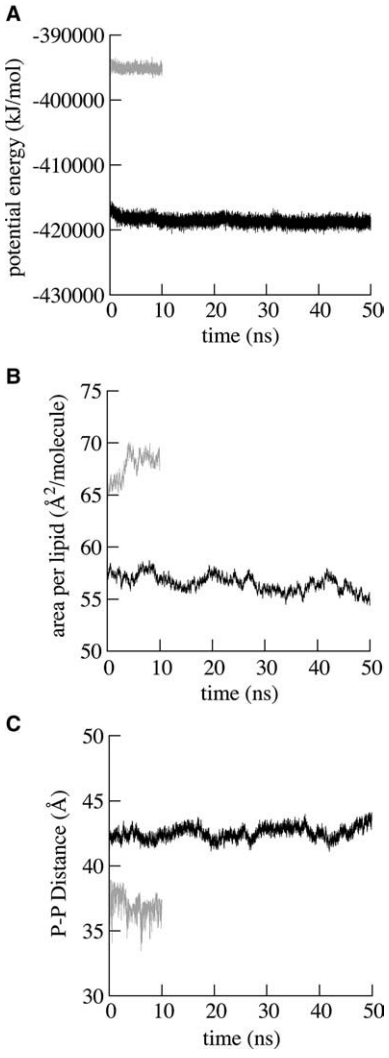

Fig. 2. Properties of the POPG (black) and POPC (gray) membranes during the course of MD simulations. (A) Potential energy of simulation systems. For ease of presentation, −150000 kJ/mol was subtracted from the POPC system potential energies on the graph. (B) Area per lipid in the simulation systems, calculated by dividing the lateral area of the simulation system by the total number of lipids in each leaflet. (C) Thickness of the lipid membranes during the simulations, measured as the distance along the membrane normal between the center of mass of phosphate atoms in the two leaflets.

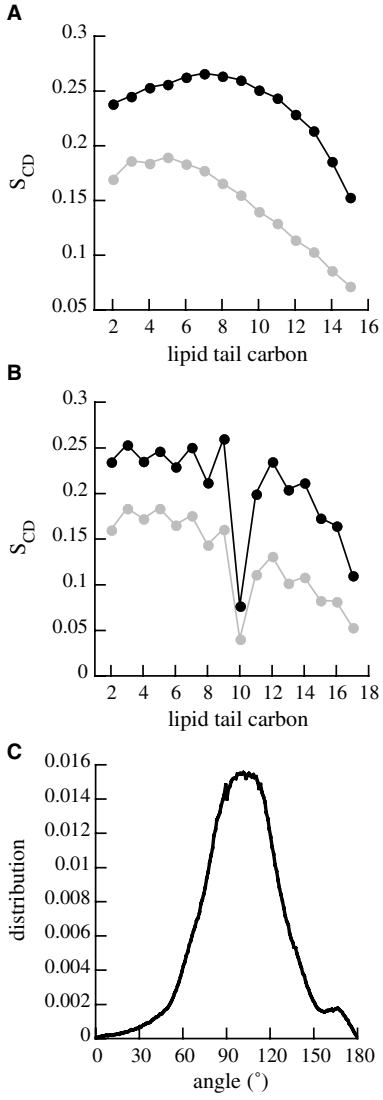

Fig. 3. (A) and (B) Deuterium order parameters (S_{CD}) for the palmitoyl (A) and oleoyl (B) chains in the POPG (black) and POPC (gray) simulations. (C) Distribution of POPG headgroup orientations measured as the angle between the membrane normal and the vector connecting P and the terminal O of POPG.

groups (Fig. 4A). As well, phosphate appears to form its strongest H-bonds with glycerol groups, although significant H-bonding occurs with water. For example, 84% of very short H-bonds (distance cutoff of $\leqslant$2.5 Å; total of 48 ± 6 H-bonds) to phosphate involve glycerol moieties. However, if we include all PG phosphate H-bonding interactions, 528 ± 11 H-bonds involve water and only 111 ± 5 H-bonds involve glycerol.

These H-bonding results for PG lipids provide useful molecular-level insights into experimental observations. Based on spectral evidence, Zhang et al. observed that the phosphate groups of PG lipids are more involved in H-bonding and polar interactions than phosphate groups in other phospholipids [28]. This is consistent with our simulations, in which PG phosphates were involved with more H-bonds than PC phosphates (640 ± 11 versus 509 ± 11). Moreover, the discussion of PG lipid H-bonding in Zhang et al. largely focused on the potential role of glycerol-phosphate H-bonds to "screen" charges between PG molecules as anionic lipids come closer together to form quasi-crystalline phases [28]. In order for the lipids to pack together in these phases, water most likely would be expelled from the phosphate region, leading glycerol groups to compensate for the lost interactions by forming more H-bonds with phosphate groups and reducing charge–charge repulsion. Similar glycerol–phosphate interactions have been proposed for PG monolayers [29], and they appear in a crystal structure of dimyristoylphosphatidylglycerol [30]. The formation of apparently very strong glycerol–phosphate H-bonds in our simulations is consistent with the potential for the glycerol groups to effectively replace water around phosphate groups. Future simulations of PG membranes under different temperature conditions could further elucidate the role of glycerol–phosphate H-bonding interactions in different membrane phases.

It is interesting to compare the H-bonding of POPG glycerol and POPS amine groups, since both are the only H-bond

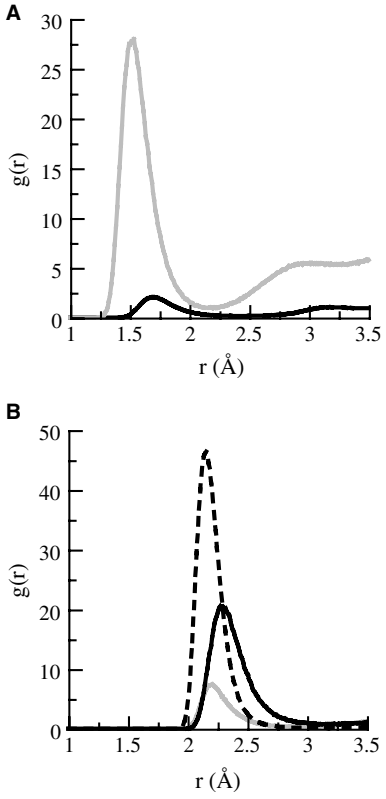

Fig. 4. Radial distribution functions (RDFs) for equilibrated POPG membranes. (A) RDFs of water (black) and glycerol H atoms (gray) from lipid phosphate O atoms. (B) RDFs of sodium cations from the O atoms of lipid phosphate (gray), glycerol (black), and ester (black dashed) groups.

donating groups in those lipids. In the POPG simulation, each glycerol forms an average of 1.63 ± 0.05 H-bonds to other lipid groups, which is strikingly similar to the extent of amine H-bonding in POPS simulations [4]. However, PS amines formed few interactions with the ester carbonyls [4,5], while POPG glycerol groups formed just under one half (≈44%) of their H-bonds with ester groups. Analogous to the differences in headgroup orientation discussed above, this likely occurs because the glycerol is uncharged and more likely than the positively charged amine to interact with groups slightly deeper in the membrane.

3.3. Ion–lipid interactions

Although sodium cations were originally placed throughout the aqueous portion of the simulation system, during the equilibration many cations rapidly approached the POPG membrane, becoming significantly embedded in the headgroup region of the bilayer (Fig. 1B and C). Interestingly, these ions seem to interact most with the ester groups next to lipid tails, not the phosphate groups (Fig. 4B). Thus, although the positively charged ions are attracted to the anionic membrane, they mostly move beyond the negatively charged phosphate groups and directly interact with polar, uncharged ester groups. Using an interaction cutoff of 3.5 Å in a cumulative RDF, the average sodium cation is coordinated to 1.12 ester O atoms but only 0.46 glycerol and 0.32 phosphate O atoms. A similar trend has been noted in other simulations with anionic PG and PS lipids [4,10].

3.4. Summary

The pure POPG membrane described in this paper appears to be well-equilibrated, and its properties are consistent with experimental data on PG membranes. Thus, this membrane provides a useful starting point to consider the interactions of membrane proteins and peptides with anionic PG membranes and compare these interactions to those that occur with zwitterionic lipids, such as PE and PC. Moreover, the similarities between lipid properties in this simulation and other simulations including PG and PS lipids provides a reassuring self-consistency between simulations of anionic lipids using different force fields and parameters. Further simulations of PG membranes, particularly in different thermal states, can give additional insights into the intermolecular interactions that underlie the properties of these membranes.

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References

[1] Ash, W.L., Zlomislic, M.R., Oloo, E.O. and Tieleman, D.P. (2004) Computer simulations of membrane proteins. Biochim. Biophys. Acta 1666, 158–189.
[2] Scott, H.L. (2002) Modeling the lipid component of membranes. Curr. Opin. Struct. Biol. 12, 495–502.
[3] Forrest, L.R. and Sansom, M.S. (2000) Membrane simulations: bigger and better? Curr. Opin. Struct. Biol. 10, 174–181.
[4] Mukhopadhyay, P., Monticelli, L. and Tieleman, D.P. (2004) Molecular dynamics simulation of a palmitoyl-oleoyl phosphatidylserine bilayer with Na^+ counterions and NaCl. Biophys. J. 86, 1601–1609.
[5] Pandit, S.A. and Berkowitz, M.L. (2002) Molecular dynamics simulation of dipalmitoylphosphatidylserine bilayer with Na^+ counterions. Biophys. J. 82, 1818–1827.
[6] Pandit, S.A., Bostick, D. and Berkowitz, M.L. (2003) Mixed bilayer containing dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylserine: lipid complexation, ion binding, and electrostatics. Biophys. J. 85, 3120–3131.
[7] Cevc, G., Ed., (1993). Phospholipids Handbook, Marcel Dekker, New York, NY.
[8] Kaznessis, Y.N., Kim, S. and Larson, R.G. (2002) Simulations of zwitterionic and anionic phospholipid monolayers. Biophys. J. 82, 1731–1742.
[9] Zaraiskaya, T. and Jeffrey, K.R. (2005) Molecular dynamics simulations and ^2H NMR study of the GalCer/DPPG lipid bilayer. Biophys. J. 88, 4017–4031.
[10] Murzyn, K., Róg, T. and Pasenkiewicz-Gierula, M. (2005) Phosphatidylethanolamine-phosphatidylglycerol bilayer as a model of the inner bacterial membrane. Biophys. J. 88, 1091–1103.
[11] Grigoriev, D., Krustev, R., Miller, R. and Pison, U. (1999) Effect of monovalent ions on the monolayers phase behavior of the charged lipid DPPG. J. Phys. Chem. B 103, 1013–1018.
[12] Cevc, G. (1991) How membrane chain-melting phase-transition temperature is affected by the lipid chain asymmetry and degree of unsaturation: an effective chain-length model. Biochemistry 30, 7186–7193.
[13] Tieleman, D.P., Sansom, M.S. and Berendsen, H.J. (1999) Alamethicin helices in a bilayer and in solution: molecular dynamics simulations. Biophys. J. 76, 40–49.
[14] Lindahl, E., Hess, B. and van der Spoel, D. (2001) GROMACS 3.0: a package for molecular simulation and trajectory analysis. J. Mol. Mod. 7, 306–317.
[15] Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A. and Haak, J.R. (1984) Molecular dynamics with coupling to an external bath. J. Chem. Phys. 81, 3684–3690.
[16] Darden, T.D., York, D. and Pedersen, L. (1993) Particle mesh Ewald: an $N\log(N)$ method for Ewald sums in large systems. J. Chem. Phys. 98, 10089–10092.

[17] Patra, M., Karttunen, M., Hyvonen, M.T., Falck, E., Lindqvist, P. and Vattulainen, I. (2003) Molecular dynamics simulations of lipid bilayers: major artifacts due to truncating electrostatic interactions. Biophys. J. 84, 3636–3645.
[18] Róg, T., Murzyn, K. and Pasenkiewicz-Gierula, M. (2003) Molecular dynamics simulations of charged and neutral lipid bilayers: treatment of electrostatic interactions. Acta Biochim. Pol. 50, 789–798.
[19] Hess, B., Bekker, H., Berendsen, H.J.C. and Fraaije, J.G.E.M. (1997) LINCS: a linear constraint solver for molecular simulations. J. Comp. Chem. 18, 1463–1472.
[20] Miyamoto, S. and Kollman, P.A. (1992) SETTLE: an analytical version of the SHAKE and RATTLE algorithms for rigid water models. J. Comp. Chem. 13, 952–962.
[21] Berger, O., Edholm, O. and Jähnig, F. (1997) Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. Biophys. J. 72, 2002–2013.
[22] Schuttelkopf, A.W. and van Aalten, D.M. (2004) PRODRG: a tool for highthroughput crystallography of protein–ligand complexes. Acta Crystallogr. D Biol. Crystallogr. 60, 1355–1363.
[23] Cowley, A.C., Fuller, N.L., Rand, R.P. and Parsegian, V.A. (1978) Measurement of repulsive forces between charged phospholipid bilayers. Biochemistry 17, 3163–3168.
[24] Lis, L.J., McAlister, M., Fuller, N., Rand, R.P. and Parsegian, V.A. (1982) Interactions between neutral phospholipid bilayer membranes. Biophys. J. 37, 657–666.
[25] Marsh, D. (1990) CRC Handbook of Lipid Bilayers, CRC Press, Boca Raton, FL.
[26] Watts, A., Harlos, K. and Marsh, D. (1981) Charge-induced tilt in ordered-phase phosphatidylglycerol bilayers evidence from X-ray diffraction. Biochim. Biophys. Acta 645, 91–96.
[27] Thurmond, R.L., Dodd, S.W. and Brown, M.F. (1991) Molecular areas of phospholipids as determined by ^{2}H NMR spectroscopy: comparison of phosphatidylethanolamines and phosphatidylcholines. Biophys. J. 59, 108–113.
[28] Zhang, Y.P., Lewis, R.N. and McElhaney, R.N. (1997) Calorimetric and spectroscopic studies of the thermotropic phase behavior of the *n*-saturated 1,2-diacylphosphatidylglycerols. Biophys. J. 72, 779–793.
[29] Dicko, A., Bourque, H. and Pézolet, M. (1998) Study by infrared spectroscopy of the conformation of dipalmitoylphosphatidylglycerol monolayers at the air–water interface and transferred on solid substrates. Chem. Phys. Lipids 96, 125–139.
[30] Pascher, I., Sundell, S., Harlos, K. and Eibl, H. (1987) Conformation and packing properties of membrane lipids: the crystal structure of sodium dimyristoylphosphatidylglycerol. Biochim. Biophys. Acta 896, 77–88.