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The structure of the zwitterionic headgroups in a DMPC bilayer as seen from Monte Carlo simulation: Comparisons with ionic solutions

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Abstract

The ionic structure of the headgroup region of a fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer is analysed on the basis of an all-atom Monte Carlo simulation. Similar analyses are also performed on simulated configurations of 2 M aqueous *o*-phosphorylcholine (*o*-PC), and 1 M, 2 M and 3 M aqueous tetramethylammonium–dimethylphosphate (TA–DP) solutions as reference systems. These homogeneous solutions contain the same ionic groups as the zwitterionic headgroup of the DMPC molecule, in the form of zwitterions and free ions, respectively. The potential of mean force acting between the pairs of these ions in water is also determined. It is found that the distribution of the tetramethylammonium (TA) and dimethylphosphate (DP) ionic groups is very similar in the headgroup region of the DMPC membrane to that in the homogeneous zwitterionic *o*-PC solution. The analysis of the potential of mean force acting between the different ion pairs in infinitely dilute aqueous solution reveals that the free energy of the formation of a contact TA or a solvent-shared DP ion pair is considerably lower than that of a contact TA–DP ion pair, due to the marked difference in the hydrophobicity of these two ions. Although this effect does not affect noticeably the structure of the TA–DP solutions investigated, it results in an unusual behaviour of the ionic groups in the systems containing zwitterions (i.e., preferential parallel alignment of the neighbouring zwitterions, contact TA–TA pair formation) that is fully in accordance with the constraints imposed by the amphiphilic structure of the lipid bilayer. This fact can explain the stability of the membranes consisting of phosphatidylcholine lipid molecules and the prevalence of these kinds of molecules among the constituents of the membrane of living cells.

Keywords: Lipid membrane; Monte Carlo simulation; Potential of mean force calculation; Ionic structure; Aqueous solutions

1. Introduction

Lipid bilayers play a role of key importance in living cells as the main constituent of the membranes separating the cells from the outside environment. Other elements of the cell membrane, such as ion channel forming proteins or transmembrane helices of signal transducting proteins are embedded in the environment of such bilayers. Therefore, detailed knowledge of the structure of lipid bilayers is essential in understanding any kind of interaction between living cells and their environment.

Besides various experimental studies, such as fluorescence [1,2], nuclear magnetic resonance (NMR) [3–8], nuclear Overhauser effect (NOESY) spectroscopy [9,10] and X-ray diffraction [11–13] measurements, which have played important roles in clarifying several features of the lipid membranes, computer simulations provide a unique opportunity to get insight into their structure at atomic level by generating full three-dimensional model configurations. The first of these simulations were performed almost two decades ago [14]. Since then, due to the rapid increase of the available computing capacity, the range of problems tackled and the type of membranes studied in this way is broadening continuously. Thus, among others, simulation studies of membranes built up by unsaturated [15–22], branching [23–27], ether-linked [27], charged [28] or fluorinated [29] phospholipid molecules, by gangliosides [30], bilayers being in non-aqueous environment [31], as well as membranes containing small dissolved molecules [32–35], cholesterol [36–47] and derivatives [48], water wire [49], lipid rafts [50], a membrane-bound protein [51,52] or a DNA segment [53] have been reported. However, most of the simulation studies are

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focused on the properties of pure membranes of saturated phospholipids with zwitterionic phosphorylcholine headgroups, such as dimyristoylphosphatidylcholine (DMPC) [54–61] or dipalmitoylphosphatidylcholine (DPPC) [62–73], the most prevalent lipid constituents of the eukaryotic cell membranes. In these bilayers the aqueous outer phase and the hydrocarbon membrane interior is separated by a rather inhomogeneous headgroup region, which is dominated by the zwitterionic phosphorylcholine groups. This headgroup region has a rather complex structure, being a mixture of positively charged tetramethylammonium (TA) and negative dimethylphosphate (DP) ionic groups, connected to each other by a covalent bond to form zwitterions, as well as water molecules and fragments of hydrophobic hydrocarbon chains. Both experimental evidences [11–13] and computer simulation results [23,27,36,37,41,42,45,48,50,52,54,59,65,67,68] show that the density of the headgroup region is considerably higher than that of both the aqueous and hydrocarbon phases. It has also been shown [25,27,43,58] that the free energy profile of some small, uncharged molecules have a sharp maximum or minimum in the headgroup region, which determines the free energy barrier these molecules have to go through when crossing the membrane. The molecular arrangement in the lipid headgroup region plays an important role in determining the structure, and thus the function of the membrane-bound protein molecules. The detailed knowledge of the atomic level structure of the headgroup region is thus of great importance in the understanding of any kind of membrane-involved biophysical processes.

Several aspects of the structure of the headgroup region of hydrated bilayers built up by phosphatidylcholine lipid molecules, such as DPPC or DMPC, have already been studied by computer simulation. Thus, it has been shown that the dipole vector of the phosphorylcholine group, approximated by the vector pointing from its P to N atom is pointing with a higher probability out of the membrane, toward the aqueous phase than toward the membrane interior [16,20,42,48,56,72]. Pasenkiewicz-Gierula et al. have investigated the hydrogen bonding interaction between water molecules and phosphorylcholine headgroups, and analysed the cross-linking of the neighbouring headgroups via bridges of hydrogen bonded water molecules [55]. They have also studied the charge associations formed by neighbouring headgroups in detail [56]. These two types of arrangement of the charged groups of the neighbouring headgroups in a lipid bilayer are the equivalent of the solvent-shared and contact ion pairs in simple aqueous solutions, respectively.

In this paper we are presenting a study of the headgroup region of a fully hydrated DMPC bilayer. The analysis, based on our earlier Monte Carlo simulation [57,60], focuses on the arrangement of the charged tetramethylammonium (TA) and dimethylphosphate (DP) groups, represented by the position of their N and P atoms, respectively. The relative arrangement of the ionic groups is characterized by their partial pair correlation functions and by the potential of the mean force acting between them. The orientation of the dipole vector of the headgroups, approximated by the vector pointing from their P to N atom (PN vector), relative to the PN vector of the neighbouring molecules as well as relative to the bilayer normal, is also discussed.

The structure of the ionic groups in the headgroup region is constrained by several factors. Thus, the zwitterionic structure of the phosphorylcholine group keeps unlike ion pairs together, and hence restricts their separation to a certain distance range. Also, due to the structure of the membrane, the tetramethylammonium and dimethylphosphate groups cannot be spread uniformly in the system, their appearance is limited to parallel layers which are overlapping with each other. In order to clarify the role of these constraints in determining the ionic structure of the lipid headgroup region, we have performed computer simulations of the aqueous solution of tetramethylammonium–dimethylphosphate (TA–DP) at different concentrations, including infinite dilution. This solution contains the same charged groups as the zwitterionic headgroup of the DMPC molecule but in the form of free ions. A Monte Carlo simulation of the aqueous solution of *o*-phosphorylcholine (*o*-PC) molecules has also been performed. Here these ionic groups are in the form of zwitterions like in the DMPC bilayer, but in a homogeneous solution. Different aspects (i.e., conformational flexibility) of the structure of this system has been analysed by Woolf and Roux [74]. A schematic representation of the solute molecules of the simulations presented here (i.e., the DMPC lipid molecule, the TA and DP ions, and the *o*-PC zwitterion) is shown in Fig. 1. In this work the TA–DP and *o*-PC solutions are regarded as reference systems, their structure is analysed in order to be compared with that of the lipid headgroup region.

2. Computer simulations

2.1. Lipid membrane simulation

A fully hydrated bilayer of DMPC molecules has been simulated in the L_α phase in the (N,p,T) ensemble by the Monte Carlo method using the code MMC [75]. The pressure and temperature of the system have been fixed at 1 atm and 310 K, respectively. The two lipid layers, containing 25 DMPC molecules each, have been surrounded by 2033 water molecules. The DMPC molecules have been described by the all-atom CHARMM22 force field optimized for phospholipids [76], whereas water has been modelled by the TIP3P potential [77]. In order to maximize the distance of two periodic images of a lipid molecule in a plane parallel to the lipid layers, a hexagonal prism-shaped simulation cell has been used. The conformation of the lipid molecules has been sampled from their torsional angle space, the bond lengths and bond angles have been kept fixed at their equilibrium values. In order to avoid simulating an infinite stack of lipid bilayers (i.e., preventing direct interaction between molecules belonging to different sides of the bilayer through the aqueous phase), lipid–water and water–water interactions have been truncated to zero at the group-based centre-centre distances of 20 Å and 12 Å, respectively. A similar cut-off combination was found to behave close to the infinite cut-off system for a hydrated DMPC monolayer [78]. Equilibration resulted in a system with the average surface area of 66.6 Å^2 per headgroup. The analyses

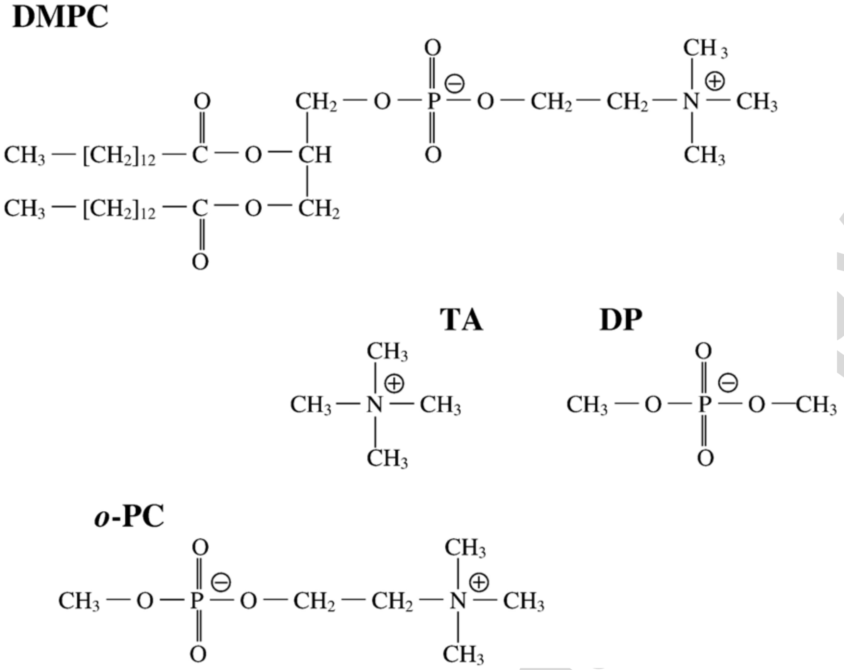

Fig. 1. Schematic representation of the dimyristoylphosphatidylcholine (DMPC) lipid molecule, the tetramethylammonium (TA) and dimethylphosphate (DP) ions, and the *o*-phosphorylcholine (*o*-PC) zwitterion.

performed are based on 1000 equilibrium sample configurations, separated by 10^5 Monte Carlo steps each. Further details of the lipid simulation are given elsewhere [57,60].

2.2. Simulation of the aqueous solutions

In order to establish appropriate reference systems for the structural analysis of the headgroups in the DMPC bilayer, we have also performed Monte Carlo simulations of the aqueous solutions of tetramethylammonium–dimethylphosphate (TA–DP) and *o*-phosphorylcholine (*o*-PC) on the (N,p,T) ensemble under the same thermodynamic conditions (i.e., 1 atm and 310 K) as those used in the membrane simulation. These systems contain the same ionic groups as the headgroup of the DMPC molecule. In the TA–DP solution these groups are present as free ions, whereas in the *o*-PC solution they are in the same zwitterionic form as in the lipid (see Fig. 1). Since the headgroups are not distributed homogeneously in the membrane, it is difficult to tell which concentration of the aqueous solution corresponds the best to the simulated membrane. Therefore, we simulated the TA–DP solution at three different concentrations. Each of the systems contained 150 ion pairs, hydrated by 7350, 3150 and 1200 water molecules in the different simulations. In the simulation of the *o*-PC solution 25 *o*-PC zwitterions have been surrounded by 525 water molecules. The solute and water molecules have been described by the same potential models as in the lipid simulation (i.e., CHARMM22 and TIP3P, respectively). The average volume of the three TA–DP solutions resulted in about 2.62×10^5, 1.37×10^5 and 0.78×10^5 Å^3, corresponding to approximately 1 M, 2 M and 3 M concentrations, respectively. In the case of the *o*-PC solution the average volume has been found to be 2.1×10^4 Å^3, corresponding to about 2 M concentration.

In the simulation of the *o*-PC solution interactions have been truncated in the same way as in the lipid simulation. This simulation has also been done with the program MMC. Every 10 water moves have been followed by a solute move, and every 600 of these moves by a volume changing move. In a water move a randomly selected molecule has been translated by no more than 0.3 Å and rotated by a maximum angle of 20° around a randomly selected space-fixed axis. 20% of the solute moves have been solute displacement steps, in which an entire zwitterion has been translated by no more than 0.1 Å and rotated around a space-fixed axis by a maximum angle of 0.5°. The remaining solute moves have been torsional angle changes, performed in the same way as in the lipid simulation [57,60]. Except for water moves, all rotations have been performed with the extension-biased method [57]. The volume changing moves have been performed by expanding or contracting the basic simulation cell in an isotropic way. The maximum change of the cell volume in one step has been set to 100 Å^3. The system has been equilibrated by 5×10^7 Monte Carlo steps. In the production phase 500 sample configurations, separated by 10^5 Monte Carlo steps each, have been saved for further evaluation.

Although we tried to do the simulations of the reference systems as similar to the original lipid simulation as possible, the truncation of the electrostatic interactions in the presence of ions, unlike in the systems containing only neutral particles, led to unacceptably large errors (since the electroneutrality of the environment a particle experiences cannot be maintained in this case). Therefore, in the simulations of the TA–DP solutions, the long-range part of the electrostatic interactions has been treated by the particle mesh Ewald method [79]. These simulations have been performed with the molecular dynamics program package GROMACS [80,81]. The temperature and pressure have been kept constant by coupling the system to an external bath [82]. All bond lengths have been kept fixed, using the SETTLE [83] and LINCS [84] algorithms for the water molecules and for the ions, respectively. The equilibration and production periods of the simulations have been 1 ns and 2 ns long, respectively. The radial distribution functions have been averaged over 10 000 equilibrium sample configurations, separated by 0.2 ps each.

2.3. Potential of mean force calculation

The potential of mean force $w(r)$ acting between two particles separated by a distance of r is related to their pair correlation function $g(r)$ through the equation

$$w(r) = -RT\ln g(r) + C, \tag{1}$$

where R is the gas constant and C is an additive constant up to which $w(r)$ is determined by $g(r)$. When calculating the potential of mean force the value of C should be chosen so that $w(r)$ approaches zero at large r values. As is evident from Eq. (1), once $g(r)$ has been determined, the calculation of $w(r)$ does not provide any additional information on the distribution of the particles in the system. However, the calculation of $w(r)$ can be important in such cases when the pair correlation function cannot be obtained directly from the simulation. Solutions of infinite dilution are examples for this kind of systems. Such solutions are usually modelled by placing two solute molecules into the simulation box, which contains an excess number of water molecules. In such systems the two solute molecules cannot provide sufficient statistics for the calculation of their pair correlation function with reasonable accuracy, especially at unfavoured distances. The potential of mean force of the solutes can, however, still be calculated by constraining their distance and calculate their excess Helmholtz free energy [85].

In order to compare the distribution of the ionic groups of the DMPC headgroup in the lipid membrane and in an infinitely dilute aqueous solution, we have calculated the potential of the mean force acting between two tetramethylammonium (TA), a tetramethylammonium and a dimethylphosphate (DP) and two dimethylphosphate ions (denoted as $w_{\mathrm{NN}}(r)$, $w_{\mathrm{NP}}(r)$ and $w_{\mathrm{PP}}(r)$, respectively) in water by Monte Carlo simulations using adaptive umbrella sampling [86,87]. These simulations have been performed in the (N,V,T) ensemble at 310 K using the program MMC. The two ions have been hydrated by 510 water molecules. The shape of the simulation box has been rectangular, the length of its edges parallel to the x, y and z axes have been set to 20 Å, 20 Å and 40 Å, respectively. Similarly to the other simulations, the ions and water have been modelled by the CHARMM22 and TIP3P force fields, respectively. One of the two ions (in the case of the TA–DP pair the TA ion) has been kept fixed in the middle of the basic simulation box, whereas the other ion has been moved along the z axis. Ion moves have been performed after every 10 water moves. (Water moves have been done in the same way as in the other Monte Carlo simulations.) In an ion move the moving ion has been rotated around a randomly chosen space-fixed axis by no more than 12.5°, and also translated by no more than 0.04 Å along the z axis.

As the translation of the moving ion was restricted to moves along the z axis, the $g(r)$ of Eq. (1) above is proportional to the $P(r)$ probability of observing the interionic distance of r. Thus, extracting $P(r)$ from a simulation would immediately yield $w(r)$. However, since $P(r)$ is an exponential function of $w(r)$, only a very limited r range can be explored this way, whereas interionic separations outside this range (unlike in the case of solutions of finite concentrations) occur only with vanishingly low probabilities making the determination of $P(r)$, and hence also that of $w(r)$ unreliable, or even impossible. The umbrella sampling methodology [88] has been developed to extend the range in which $P(r)$ can be properly sampled by adding an extra function $Q(r)$ to the energy used in the Boltzmann factor, and correct the results *a posteriori*. Since the optimal choice for $Q(r)$ is exactly $-w(r)$ (i.e., in this case $P(r)$ is uniform), this leads to an iterative scheme called adaptive umbrella sampling, where the initial estimate for $Q(r)$ comes from the estimate of $w(r)$ on an initially sampled range, and successive iterations (whose result is obtained from a steadily improving $Q(r)$) can sample larger and larger distance ranges [86,87].

In this way, the potential of mean force acting between the two ions, averaged over all possible orientations of the moving ion, has been obtained. However, since the other ion has been kept fixed in the simulation, the resulting $w(r)$ functions are depending on the orientation of the fixed ion relative to the vector joining the two ions (i.e., the space-fixed z axis). Since the tetramethylammonium ion is nearly spherical, this fact

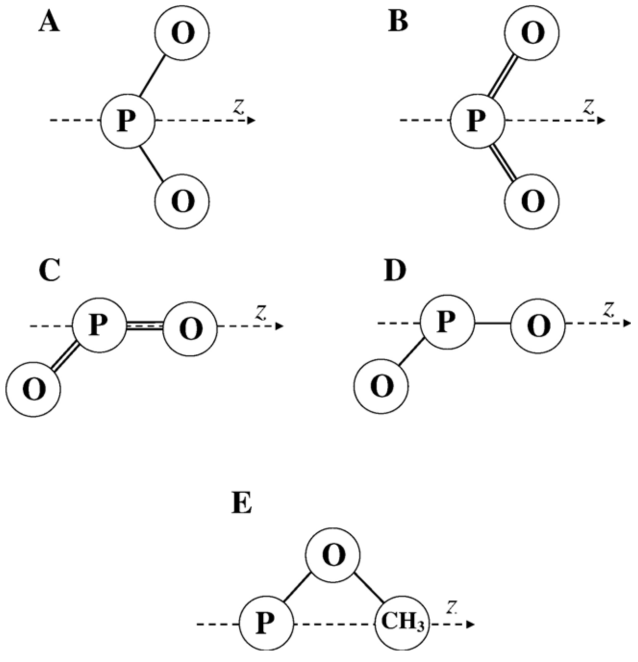

Fig. 2. Different orientations of the fixed central DP ion relative to the space fixed z axis (i.e., the vector pointing from this ion toward the moving DP ion), used in the calculations of the potential of mean force acting between two DP ions in an infinitely dilute aqueous solution.

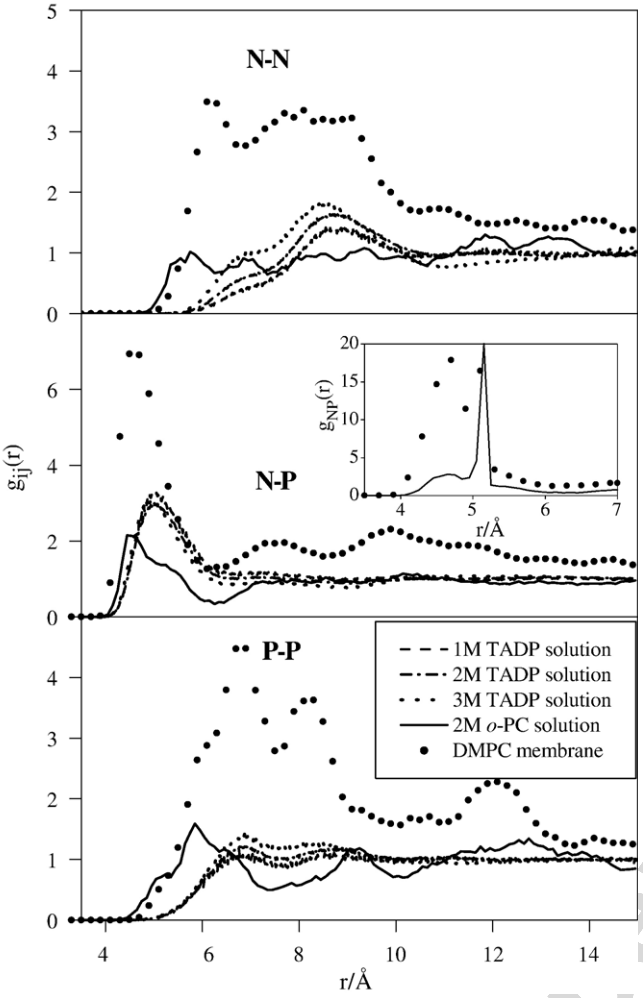

Fig. 3. Partial pair correlation functions of the TA and DP ionic groups, represented by their N and P atoms, respectively, in 1 M (dashed lines), 2 M (dash-dotted lines) and 3 M (dotted lines) TA–DP solutions, in 2 M *o*-PC solution (solid lines) and in the DMPC membrane (full circles). The $g_{NP}(r)$ functions of the zwitterionic solution and the DMPC membrane do not contain intramolecular contribution. The $g_{NP}(r)$ functions containing both intra- and intermolecular terms are shown in the inset.

results only in minor inaccuracies in the obtained $w(r)$ function of the TA–TA and TA–DP pairs. (In these simulations the central TA ion has been oriented in such a way that one of its twofold symmetry axes coincides with the space-fixed z axis.) On the other hand, the resulting $w(r)$ function of the DP–DP pair depends largely on the orientation of the fixed central ion. Therefore, we calculated the $w_{PP}(r)$ function at five different orientations of the central dimethylphosphate ion. The direction of the space-fixed z axis relative to the orientation of the central DP ion is shown in Fig. 2 for the five orientations considered.

In order to obtain a reasonable convergence, each $w(r)$ function has been determined in smaller (i.e., 1.2 Å–2.0 Å wide) r segments. Therefore, each $w(r)$ function has been determined by a set of 3–5 simulations, performed at different but overlapping r ranges. The individual segments of $w(r)$ have been joined together by fitting them to each other at the overlapping part of their r ranges.

In the equilibration phase of the simulations 2.5×10^7 Monte Carlo steps have been done. The $w(r)$ function has then been calculated in 100 iteration steps, each of them containing 5×10^5 Monte Carlo moves.

3. Results and discussion

3.1. Partial pair correlation functions

The partial pair correlation functions of the N atoms of the TA and P atoms of the DP ionic groups are shown on Fig. 3 in 1 M, 2 M and 3 M TA–DP as well as in the 2 M *o*-PC solution and in the DMPC membrane, as a result of our simulations. It should be noted that due to the inhomogeneous distribution of the N and P atoms in the lipid membrane, the magnitude of the peak height of the $g(r)$'s has little physical relevance in this system. The reason for this is that, by definition, the partial pair correlation function of the i and j type atoms can be given as

$$g_{ij}(r) = \frac{\rho_{ij}(r)}{\rho_j}, \tag{2}$$

where $\rho_{ij}(r)$ is the density of the j type atoms at a distance of r from the central atom of type i, and ρ_j is the average density of the j type atoms in the entire system. Since in the DMPC membrane the N and P atoms are located only in a limited region of the system, their overall density in the entire simulation box is of little meaning, and through Eq. (2) this arbitrariness is also reflected in the magnitude of their partial pair correlation functions. Such arbitrariness can largely be avoided when characterizing the peaks of the $g(r)$'s by their coordination number, defined as

$$c_{ij}(r) = 4\pi\rho_j\int_0^r R^2 g_{ij}(R)\mathrm{d}R = 4\pi\int_0^r R^2\rho_{ij}(R)\mathrm{d}R, \tag{3}$$

instead of their height. In this way, the intensity of the peaks of the $g(r)$'s becomes comparable in the different systems. The position of the maxima and minima as well as the coordination number of the peaks (integrated up top the following minima) of the $g_{ij}(r)$ functions obtained in the 2 M TA–DP and *o*-PC

Table 1
Position of the minima and maxima of the N–N, N–P and P–P partial pair correlation functions in the DMPC membrane as well as in the 2 M *o*-PC and 2 M TA–DP solutions, as obtained from our simulations

		1st peak	1st minimum	2nd peak	2nd minimum
NN	DMPC membrane	6.2	6.8 (0.85)	8.2	10.5 (5.0)
	2 M *o*-PC solution	5.8	6.3 (0.5)		
	2 M TA–DP solution	7.0	7.4 (0.5)	8.7	10.9 (5.5)
NP	DMPC membrane	4.6	6.2 (2.1[a])	7.5	8.5 (3.3[a])
	2 M *o*-PC solution	4.5	6.3 (2.0[a])		
	2 M TA–DP solution	5.0	6.5 (1.7)	7.4	9.0 (3.7)
PP	DMPC membrane	6.8	7.5 (1.7)	8.2	10.1 (4.3)
	2 M *o*-PC solution	5.9	7.4 (1.4)	9.2	10.1 (4.1)
	2 M TA–DP solution	6.9	7.6 (1.2)	8.7	10.1 (4.1)

Distances are in Ångström (Å). Values in parenthesis are the coordination numbers corresponding to the given distance.

[a] Value includes the intramolecular contribution.

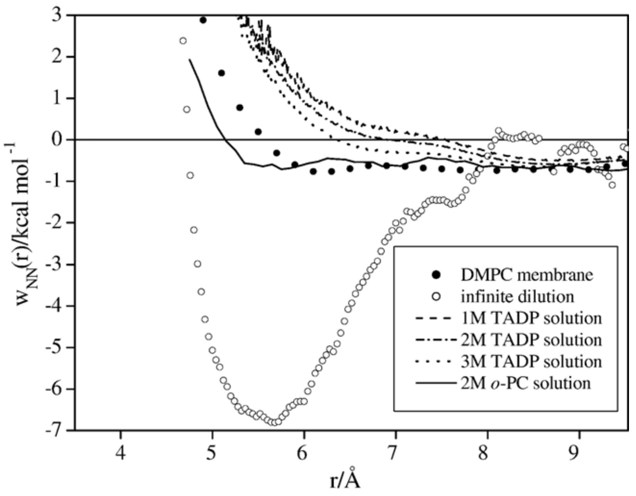

Fig. 4. Potential of the mean force acting between two TA ionic groups in infinitely dilute aqueous solution (open circles), in 1 M (dashed line), 2 M (dash-dotted line) and 3 M (dotted line) TA–DP solutions, in 2 M *o*-PC solution (solid line) and in the DMPC membrane (full circles).

solutions as well as in the DMPC membrane are summarized in Table 1.

The overall behaviour of the resulting partial pair correlation functions is rather similar in the different systems studied. This similarity is reflected both in the shape and the characteristic values (i.e., position of minima and maxima, coordination number) of the $g(r)$'s. In the membrane, the $g_{NP}(r)$ function (i.e., the pair correlation function of the unlike ions) has a sharp, well defined first peak at about 4.6 Å, where the other two $g(r)$'s are still zero. As seen in the inset of Fig. 3, the distance at which this peak, corresponding to the contact ion pairs, is located is even smaller than the preferred intramolecular N–P separation of 5.1 Å. Similar behaviour is observed in the 2 M *o*-PC solution, indicating that the chain connecting the two ionic groups to form a zwitterion is not long and flexible enough to let the two groups as close to each other as their contact distance. The position of the first peak of the TA–DP ionic solutions agrees also well with that of the DMPC membrane. The main difference between the $g_{NP}(r)$ functions obtained in the DMPC bilayer and in the homogeneous solutions is that in the membrane the second and even the third N–P coordination shell can also clearly be identified at 7.7 Å and 9.9 Å, respectively, whereas in the *o*-PC and TA–DP solutions the $g_{NP}(r)$ function becomes nearly uniform beyond the first peak (although in the ionic solutions a very slight second peak can still be detected, at about the same position as in the DMPC membrane). This difference clearly reflects structural correlations imposed by the planar packing of the lipid molecules in the membrane.

In the DMPC membrane the $g(r)$'s of both like ion pairs have a rather broad first bump, split into two separate peaks at much larger distances (i.e., between about 6 Å and 10 Å) than that of the first peak of $g_{NP}(r)$. In the case of $g_{PP}(r)$ the first of these two peaks, located at 6.8 Å corresponds to the solvent-shared ion pairs, connected through a bridging water molecule that forms a hydrogen bond with both ions [56], whereas the second peak at 8.2 Å is due to the solvent-separated pairs. Both peaks are clearly present in the homogeneous solutions, as well. On the other hand, the first peak of the $g_{NN}(r)$ function in the DMPC membrane at 6.2 Å is due to the contact pairs of the bulky TA ionic groups. The formation of such contact pairs of the unlike ionic groups is facilitated by the hydrophobic nature of the TA ions [89]. This peak is also present in the *o*-PC solution, however, the $g_{NN}(r)$ functions of the TA–DP solutions lack it (although at high enough concentrations a shoulder appears on the low *r* side of the first, already solvent-separated peak of these $g(r)$'s, still at considerably larger distances than in the zwitterionic systems). This finding indicates that the contact pair formation of the positively charged TA ionic groups is facilitated by the fact that they are chemically bound to a negatively charged DP ionic group forming a zwitterion.

The comparison of the $g_{PP}(r)$ and $g_{NN}(r)$ functions of the different systems shows again, similarly to that of $g_{NP}(r)$'s that the correlation between the ions is considerably longer in the lipid membrane than in the homogeneous solutions. Furthermore, the first peak of both $g_{PP}(r)$ and $g_{NN}(r)$ appears at somewhat smaller distances in the *o*-PC solution than in the membrane. These findings indicate again the constraining effect of the planar packing of the DMPC molecules in the membrane.

3.2. Potentials of the mean force

The potentials of the mean force acting between two TA, a TA and a DP, and two DP ions or ionic groups in the systems simulated, including infinitely dilute solutions, are shown on Figs. 4–6, respectively. As is seen from these figures, the comparison of the $w(r)$ instead of the $g(r)$ functions stresses the large similarity between the ionic structure of the headgroup region of the DMPC bilayer and the homogeneous systems, in particular, the zwitterionic *o*-PC solution. The position as well as the amplitude of the peaks and wells of all the three $w(r)$ functions of these two systems agree very well with each other. The only exception is that the two minima of $w_{PP}(r)$ are further apart in the homogeneous solution than in the membrane,

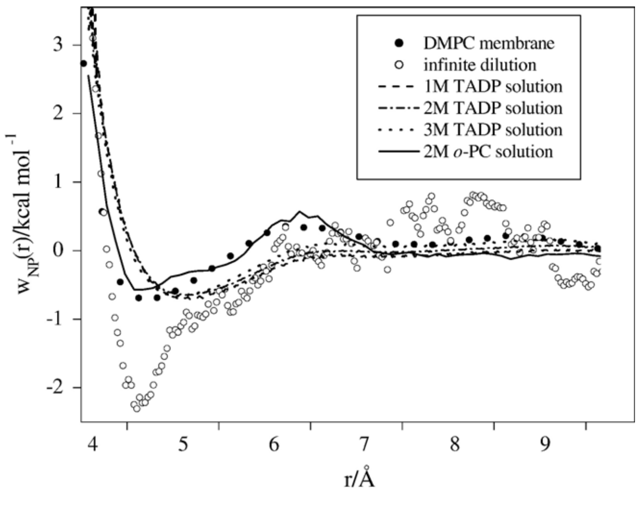

Fig. 5. Potential of the mean force acting between a TA and a DP ionic group in infinitely dilute aqueous solution (open circles), in 1 M (dashed line), 2 M (dash-dotted line) and 3 M (dotted line) TA–DP solutions, in 2 M *o*-PC solution (solid line) and in the DMPC membrane (full circles).

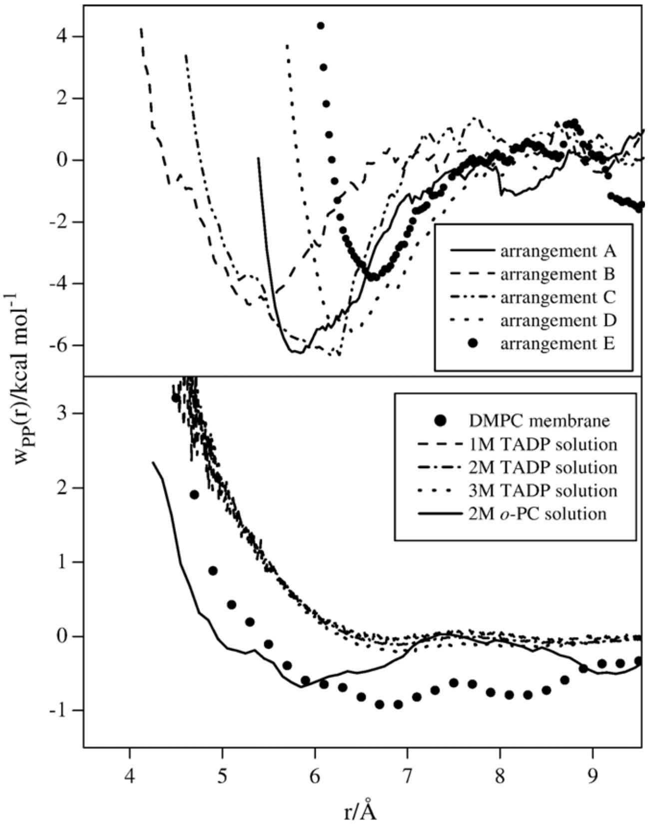

Fig. 6. Top: potential of the mean force acting between two DP ionic groups in infinitely dilute aqueous solution in arrangements A (solid line), B (dashed line), C (dash-dotted line), D (dotted line), and E (full circles). (For the different arrangements, see Fig. 2.) Bottom: potential of the mean force acting between two DP ionic groups in 1 M (dashed line), 2 M (dash-dotted line) and 3 M (dotted line) TA–DP solutions, in 2 M *o*-PC solution (solid line) and in the DMPC membrane (full circles).

although the position of the barrier separating them coincides in the two systems.

As is seen from Fig. 4, the $w(r)$ of the TA ion pair at infinite dilution is similar in many respects to the potential of the mean force acting between these ionic groups in the zwitterionic systems, having their first minimum at about the same distance. This minimum corresponding to the contact ion pair is much deeper at the infinitely dilute system than in the DMPC membrane or in the *o*-PC solution of finite concentrations, since many-body correlations between the solvated ions make other interionic separations also less unfavourable. Similarly, the shape of the $w_{NP}(r)$ function of the infinitely dilute solution is rather close to that of the systems containing zwitterions (see Fig. 5). In particular, all of these functions have a well-defined contact pair minimum at 4.6 Å. These functions can explain the behaviour of the TA and DP ionic groups in the different systems. Although the $w(r)$ functions are only determined up to an additive constant by the distribution of the particles in the system (see Eq. (1)), and thus their absolute value can only be estimated in the simulations from the requirement that at infinitely large distances they should be equal to zero, it is still evident from Figs. 4 and 5 that the first (contact) minimum of the $w(r)$ of two TA ions is much deeper than that of a TA and a DP ion at infinite dilution. This surprising finding can again be explained by the difference in the hydration of the TA and DP ions. Namely, the negatively charged DP ion is strongly hydrophilic, and can even form several strong hydrogen bonds as a H-acceptor with the water molecules of its first coordination shell. On the other hand, the positively charged TA ions are hydrated in a hydrophobic way [89]. This difference in the hydration behaviour of the two ions could, in principle, locally even overcompensate the electrostatic repulsion of the like ion pairs in solutions of finite concentrations leading to the formation of unusual microstructure of their solution (the theoretical background of the possibility of such unusual ionic arrangements in the presence of a specific interaction acting against simple electrostatics has been described in 2 dimensions by di Caprio et al. [90]). However, in the TA–DP solutions studied here this specific effect, originated in the different hydrophobicity of the two ions, is not found to be strong enough for noticeably modify the arrangement of the ions imposed by their simple charge–charge interactions. In other words, the 4–5 kcal/mol difference between the free energy of formation of the TA–TA and TA–DP contact pairs, observed at infinite dilution is not enough to the appearance of a noticeable amount of contact TA ion pairs even in the 1 M TA–DP solution because of the strong repulsion due to the large enough density of the positive charges. Nevertheless, the ability of the TA ions of forming contact pairs is evident, and this ability is realized in the zwitterionic systems, where the oppositely charged DP groups are constrained to be at the vicinity of the TA groups, and hence can mediate their electrostatic repulsion.

The comparison of the $w_{PP}(r)$ functions is also consistent with the above picture. The depth of the free energy well corresponding to the solvent-shared DP pairs at infinite dilution roughly equals to that of the contact TA pairs (see Figs. 4 and 6). The analysis of $w_{PP}(r)$ functions at infinite dilution can also give some information on the preferential orientation of the DP ion pairs in these systems. The position of the main minimum of $w_{PP}(r)$ agrees in the zwitterionic *o*-PC solution with that of arrangements B and C, i.e., when the central ion turns toward the other ion with one or two of its double bonded O atoms in the infinitely dilute system. These arrangements can easily correspond to structures in which the two DP ions are connected by a “bridging” water molecule, which forms a hydrogen bond with both of the ions. The two arrangements in which the central ion turns toward the other ion with one of its methyl groups (i.e., D and E) do not play an important role in forming the interionic structure of the DP ions in this solution. A different picture can, however, be obtained from the comparison of the $w_{PP}(r)$ function of the DMPC membrane with the results of the different DP ion arrangements at infinite dilution. The first minimum of the $w_{PP}(r)$ of the lipid bilayer is closer to that in arrangements D and E than that in the other three arrangements. This finding can again be explained by the fact that in the membrane the distribution of the P atoms reflects the packing of the lipid molecules themselves.

3.3. Relative orientation of the PN vectors

The cosine distribution of the angle γ formed by the PN vector of two neighbouring DMPC molecules in the membrane is shown on Fig. 7. (The advantage of using cosine distribution

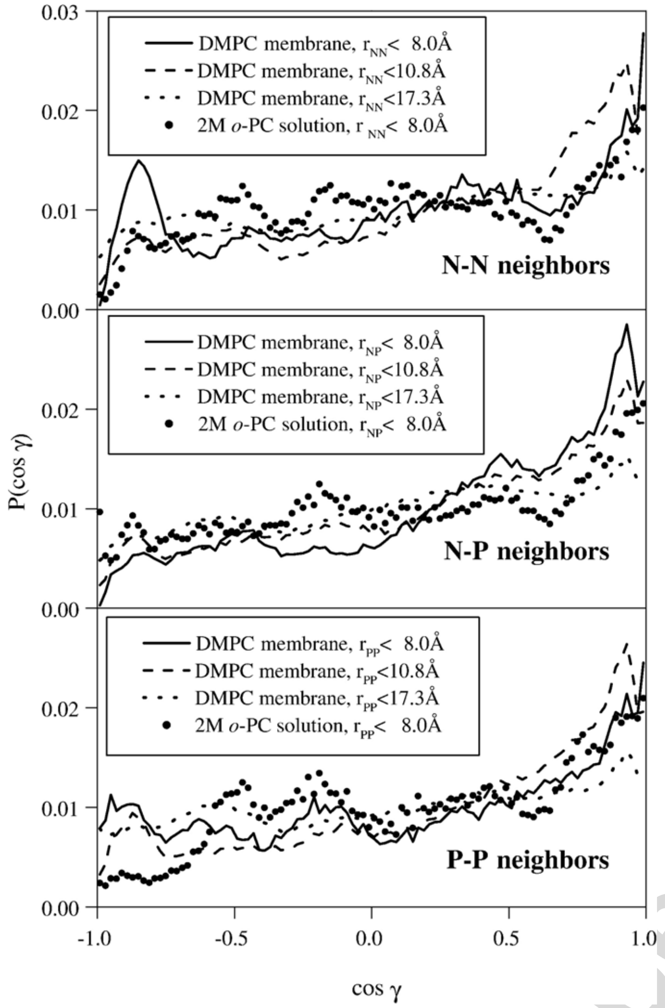

Fig. 7. Cosine distribution of the angle γ formed by the PN vector of two neighbouring molecules in the DMPC membrane. The distance of the neighbours is defined as the distance of their N atoms (top), closest N and P atom (middle) and P atoms (bottom). Neighbours within 8.0 Å (solid lines), 10.8 Å (dashed lines) and 17.3 Å (dotted lines) are taken into account. These values correspond approximately to the coordination number values of 2, 5 and 15, respectively. The $P(\cos\gamma)$ distributions of the neighbours within 8.0 Å, obtained in the 2 M *o*-PC solution, are also shown (full circles).

instead of the distribution of the angle γ itself is that uniform distribution of the neighbour orientations corresponds to a constant $P(\cos\gamma)$ distribution and to a $P(\gamma)$ distribution proportional to $\sin\gamma$. This allows a much more straightforward interpretation of the resulting cosine than angular distribution.) In deciding whether two molecules are neighbours, we have used three different definitions. Thus, according to the first, second and third definition, two DMPC molecules are regarded as neighbours if the distance of their N atoms, the shorter of the two distances of their N and P atoms, or the distance of their P atoms, respectively, is shorter than a given limiting value. Molecular pairs satisfying these definitions are referred to here as N–N, N–P and P–P neighbours, respectively. In this analysis we have used three different limiting distance values, i.e., 8.0 Å, 10.8 Å, and 17.3 Å. These distances correspond roughly to the coordination number values of 2, 5, and 15, respectively, for all the three atom pairs. For comparison, the $P(\cos\gamma)$ distribution of the N–N, N–P and P–P neighbours within 8.0 Å has also been calculated in the 2 M *o*-PC solution. The resulting distribution functions are also plotted on Fig. 7.

As is seen from Fig. 7, the obtained $P(\cos\gamma)$ distribution functions are rather similar for N–N, N–P and P–P neighbours. In every case there is a weak but clear preference of the neighbouring PN vectors for the parallel alignment. This preference is about as strong for the nearest two as for the nearest five neighbours (i.e., for neighbours within 8.0 Å and 10.8 Å), whereas it becomes considerably weaker when more distant neighbours, located between 10.8 Å and 17.3 Å, are also taken into account. Moreover, the preference for parallel alignment is roughly equally strong among N–N, N–P and P–P neighbours. This finding indicates that head-to-tail arrangements of the PN vectors do not play a dominant role in the lipid headgroup structure, because the preference of the nearest N–P neighbours would otherwise be much stronger for parallel PN vector alignment than that of the nearest N–N or P–P neighbours.

Besides the main peak of all the calculated $P(\cos\gamma)$ functions, the distribution of the N–N neighbours corresponding to the shortest limiting distance (i.e., 8 Å) has another well defined peak just above −1. The $P(\cos\gamma)$ function of the nearest P–P neighbours also has a similar, although smaller peak, whereas such a peak is completely missing for N–P neighbours. This finding indicates that, besides their main preference for parallel alignment, the neighbouring PN vectors prefer also head-to-head and tail-to-tail arrangements.

These results seem somewhat surprising, since the PN vector joins the centre of the positively and negatively charged groups of the zwitterionic lipid headgroup, and thus coincides approximately with its dipole vector. Therefore, on the basis of only electrostatic interactions, one would expect either preferential antiparallel or head-to-tail nearest neighbour arrangement of the PN vectors. On the other hand, the observed preferential parallel orientation is consistent with the inhomogeneous structure of the membrane, in which the N atoms are located, on average, closer to the aqueous phase than the P atoms [16,19,36–39,42,28,54,59,65,68], and hence the PN vectors prefer to point

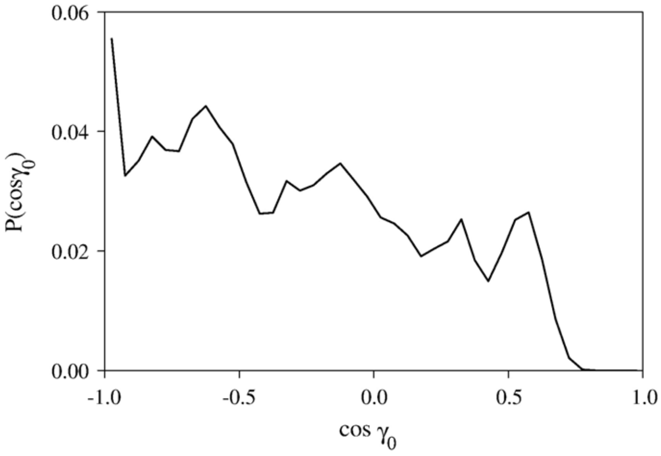

Fig. 8. Cosine distribution of the angle γ_0 formed by the PN vector of the DMPC molecules with the bilayer normal vector, pointing toward the middle of the membrane.

toward the aqueous phase rather than the membrane interior [16,20,42,48,56,72]. This is demonstrated in Fig. 8, which shows the cosine distribution of the angle γ_0 formed by the bilayer normal vector, pointing toward the middle of the membrane, and the PN vector of a DMPC molecule. The preference of the PN vectors for pointing toward the aqueous phase is evident. This orientational ordering effect of the membrane on the PN vectors results also in a preference for parallel orientation of the PN vectors with each other. On the other hand, the observed head-to-head and tail-to-tail orientations are probably preferred by those molecules whose PN vector is almost perpendicular to the bilayer normal, and hence lie in a plane roughly parallel to the bilayer. Nevertheless, it is still surprising that these molecules prefer head-to-head and tail-to-tail orientations instead of the electrostatically more reasonable head-to-tail arrangement. The reason for this behaviour is probably originated in the hydrophobic hydration of the TA ions [89].

In view of the above findings it is very interesting to compare the $P(\cos\gamma)$ distributions of the nearest neighbour molecules in the *o*-PC solution with the similar functions obtained in the DMPC membrane. As seen in Fig. 7, these functions are remarkably similar to each other, indicating that, in spite of the lack of any external ordering effect in the homogeneous solution of the *o*-PC zwitterions, the orientational structure of the PN vectors here is rather similar to that in the headgroup region of the DMPC bilayer. This means that the preferential parallel orientation of the PN vectors in the membrane is not an unfavourable arrangement forced by the structure of the bilayer, but it is the consequence of the interactions of the ionic groups, including the interplay between the hydrophobic nature of the TA and hydrophilic nature of the DP groups.

4. Conclusions

The present comparison of the ionic structure of the headgroup region of a fully hydrated DMPC bilayer with that of homogeneous solutions containing the same ionic groups in the form of free ions as well as zwitterions has shed some light to the origin of the observed arrangement of these ionic groups in the membrane. Thus, it has clearly been seen from the comparison of the $g_{ij}(r)$ and $w_{ij}(r)$ functions that the distribution of the ionic groups in the membrane is very similar to that in the zwitterionic *o*-PC solution. The analysis of the potential of the mean force acting between two TA ions, and a TA and a DP ion in water has shown that although the ions form favourably a contact pair in both cases, the corresponding free energy minimum is considerably deeper in the case of the two TA ions than for the TA–DP ion pair. Similarly, the free energy of the formation of a solvent-shared DP ion pair is also lower than that of the formation of a contact TA–DP pair. Although this effect, caused by the marked difference in the hydrophobicity of these two ions is certainly not enough to make the interionic structure of the homogeneous TA–DP solutions of at least 1 M concentration noticeably different from the usual structure of simple ionic solutions, the ability of the TA ions of forming contact pairs is realized in the zwitterionic systems, including the DMPC membrane. This effect is also responsible for the rather unusual preferential parallel alignment of the PN vector of the neighbouring molecules in the zwitterionic systems. On the other hand, such a parallel orientation of the PN vectors is imposed by the structure of the membrane itself for the DMPC headgroups. Hence, the structural constraints of the DMPC bilayer on the ionic arrangement of the headgroup region are fully in accordance with the rather unusual properties of the ionic groups building up the headgroup. This fact can explain the remarkable stability of the phosphatidylcholine membranes and the prevalence of the phosphatidylcholine lipid molecules among the constituents of the membrane of living cells.

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