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Molecular dynamics simulations of glyphosate in a DPPC lipid bilayer☆

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ABSTRACT

Extensive molecular dynamics simulations have been performed to study the effect of glyphosate (in their neutral and charged forms, GLYP and $GLYP^{2-}$, respectively) on fully hydrated DiPalmitoylPhosphatidylCholine (DPPC) lipid bilayer. First, we calculated the free energy profile (using the Umbrella Sampling technique) for both states of charge of glyphosate. The minimum value for the free energy for GLYP is $\sim -60\,kJ\,mol^{-1}$ located at $z = \pm 1.7\,nm$ (from the lipid bilayer center), and there is almost no maximum at the center of the lipid bilayer. By contrast, the minimum for $GLYP^{2-}$ is $\sim -35\,kJ\,mol^{-1}$ located at $z = \pm 1.4\,nm$ (from the lipid bilayer center), and the maximum reaches $\sim 35\,kJ\,mol^{-1}$ at the center of the lipid bilayer. Then, different lipid bilayer properties were analyzed for different glyphosate:lipid (G:L) ratios. The mean area per lipid was slightly affected, increasing only 5% (in the presence of glyphosate at high concentrations), which is in agreement with the slight decrease in deuterium order parameters. As for the thickness of the bilayer, it is observed that the state of charge produces opposite effects. On one hand, the neutral state produces an increase in the thickness of the lipid bilayer; on the other, the charged form produces a decrease in the thickness, which not depend linearly on the G:L ratios, either. The orientation of the DPPC head groups is practically unaffected throughout the range of the G:L ratios studied. Finally, the mobility of the lipids of the bilayer is strongly affected by the presence of glyphosate, considerably increasing its lateral diffusion coefficient noteworthy (one order of magnitude), with increasing G:L ratio.

1. Introduction.

The importance of herbicides in agriculture is well known. Its use allows for the preservation of the moisture and the nutrients of the soil to improve productivity, together with a reduction in the use of fertilizers. Glyphosate stands out among the most commonly used herbicide formulations. It is a broad-spectrum, systemic herbicide used both in the pre-planting phase, and in the pre-harvest stage of herbicide-tolerant and conventional crops to control annual broad-leaf weeds. Previous studies (Arregui et al., 2003; Krüger et al., 2014) have shown that it is possible to find residues of glyphosate in stems or leaves at the harvest stage. However, the concentration of glyphosate found strongly depends on the application method employed for such herbicides. In this way, it is possible for mammals to ingest glyphosate through the feeding of such crops. Even though the toxicity of glyphosate has been extensively studied in several organisms, there is still some controversy about the real effects of glyphosate on human health. Some studies (Wester et al., 1991; Williams et al., 2000; Kier and Kirkland, 2013) state that there is no risk associated with specific glyphosate exposure, while others (Richard et al., 2005; Gasnier et al., 2010; Saes et al., 2010) claim that exposure to glyphosate may pose a health risk, depending on various factors, such as formulation type, concentration, and time of exposure. As it can be seen, the mechanism of action of the herbicide is a complex phenomenon, and a key step in understanding it is the study of its fundamental interactions with the bio-membrane (Seydel et al., 1994; van Balen et al., 2004; Boggara and Krishnamoorti, 2010).

Cell membranes can be studied by molecular dynamics (MD) simulation technique, as it has been shown in a large number of previous works (López Cascales, 1996; López Cascales and Huertas, 1997; Tieleman et al., 1997; Ash et al., 2004). This technique also allows for the calculation of the partition of small solutes from the bulk solution to the interior of the lipid bilayer, through Umbrella Sampling (Torrie and Valleau, 1977), thus obtaining the Potential of Mean Force (MacCallum and Tieleman, 2006; MacCallum et al., 2007, 2008; Porasso et al., 2009; López Cascales et al., 2011). This kind of study also allows for

☆ Glyphosate interaction with DPPC lipid bilayer.

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determining different static and dynamic properties of lipid bilayer in the presence of the solute.

The potentiometric titration study of glyphosate (Sprankle et al., 1975) shows that this herbicide has four different ionization constants: $pK_{a1} = 0.8$, $pK_{a2} = 2.6$, $pK_{a3} = 5.6$ and $pK_{a4} = 10.6$. As a consequence, glyphosate is usually in ionic form, generally with a negative net charge on the pH scale above 2.6, due to the loss of protons from the carboxylic and phosphoric groups. An exception is possible in a narrow pH range, in acid medium, where glyphosate can be found with neutral net charge (at pH 0.8–2.6) or positive charged (at pH < 0.8) due to the protonation of the amino group. Given the various charge states of glyphosate and the existence of different pH values throughout the digestive system of mammals, two states of charge for glyphosate are proposed to be studied: (i) the neutral state (hereinafter called GLYP), which corresponds to pH ~ 2 (stomach of mammals), and (ii) the cahrged form (hereafter called $GLYP^{2-}$) with an excess of two negative charges, corresponding to the large intestine, where pH ~ 8.

In the present study, a series of MD simulations of systems composed of a DiPalmitoylPhosphatidylCholine (DPPC) lipid bilayer in the fluid phase, glyphosate (for two different states of charge), and water molecules were performed in order to study the interaction between glyphosate and the DPPC lipid bilayer. First, we performed a simulation using the Umbrella Sampling technique in order to obtain the free energy profile and some properties of the system. And secondly, we conducted an unbiased MD simulation by varying the concentration of glyphosate in order to examine the effects of the herbicide in the DPPC structural properties, such as area per lipid, thickness, deuterium order parameter, translational diffusion coefficient, and radial distribution function.

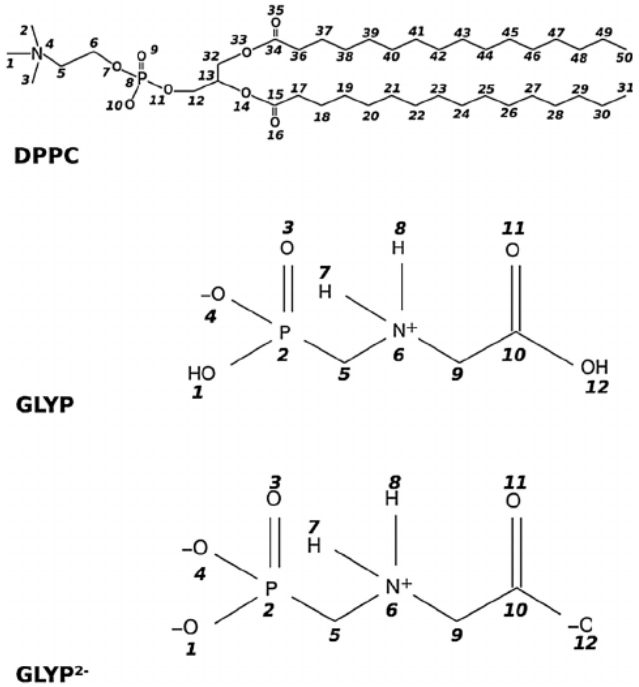

Fig. 1. Chemical structure and atomic numeration of DPPC and for glyphosate (N-(phosphonomethyl)glycine, CAS 1071-83-6): GLYP corresponds to neutral form, and $GLYP^{2-}$ is the charged form.

2. Methodology

2.1. Simulation details

Molecular dynamics (MD) simulations were performed using the GROMACS 3.3.3 (Berendsen, 1995; Lindahl and Hess, 2001) program suite. For the initial lipid bilayer structure, a well equilibrated system containing 72 DPPC (López Cascales and Hernández Cifre, 1998) lipids and approximately 2650 water molecules were used. The lipid molecules (Egberts et al., 1994) and the herbicide molecules (Schuttelkopf and Aalten, 2004) were modeled using the GROMOS force field and the water molecules were modeled by the SPC (Single Point Charge Berendsen et al., 1981) model. Both the coordinates and the partial charge of the herbicides molecules were generated using the PRODRG (Schuttelkopf and Aalten, 2004), which is based on the GROMOS force field and generates partial charges based on the United Atom model (Fig. 1). MD simulations were performed under conditions of constant pressure and temperature (NPT), by coupling to an isobaric and isothermal bath, proposed by Berendsen et al. (1984), with a coupling constant $\tau_T = 0.1$ ps and $\tau_P = 1.0$ ps for temperature and pressure, respectively. All simulations were implemented at a temperature of 350 K and a pressure of 1 atm. This temperature was chosen in order to ensure that the system was in the liquid crystalline state (biologically relevant), considering that the transition temperature of DPPC is 314 K (Seelig and Seelig, 1974). A cut-off of 1 nm was set for Lennard–Jones interactions and electrostatic interactions were evaluated using the method of particle mesh Ewald (Essmann et al., 1995; Darden et al., 1993), using real space interactions cut-off of 0.9 nm. All simulations were performed with a step of 2 fs integration. At the beginning of each simulation, a steepest descent minimization process was applied to the complete system, in order to remove any excess of strain due to an overlap of neighbor atoms.

2.2. Free energy profile

The free energy resulting from partitioning the herbicides from the bulk water to the interior of the lipid membrane was calculated using the Potential of Mean Force (PMF) method, applying the Umbrella Sampling (Torrie and Valleau, 1977) technique, which has proven to be a suitable tool for this type of systems (MacCallum and Tieleman, 2006; MacCallum et al., 2007, 2008; Porasso et al., 2009; López Cascales et al., 2011). In this sense, two glyphosate molecules were dragged along the z-axis (defined perpendicularly to the water-lipid interphase) by applying a harmonic potential of 3000 kJ mol^{-1} nm^{-2}, which allows for a free movement in the x–y plane. The relation between the free energy and the PMF can be expressed as:

$$\Delta G(z) = -\mathrm{RT} \ln \frac{C_h^{eq}(z)}{C_h^*} = -\mathrm{RT}\ \mathrm{PMF}(z) \quad (1)$$

where $\Delta G(z)$ represents the free energy profile along the z-axis, RT is the constant of the gases and temperature, $C_h^{eq}(z)$ is the concentration profile of the herbicide along the z-axis, C_h^* represents the concentration of herbicide in the solution and PMF(z) is recovered using the Weighted Histogram Analysis Method (WHAM) (Kumar et al., 1992). As explained in the Introduction, due to the multiple states of charge of the glyphosate, two cases were examined by MD simulations, namely neutral glyphosate (GLYP) and charged glyphosate ($GLYP^{2-}$). Net charges of 0 and −2 for neutral and charged glyphosate were use to represent the situation in the stomach and in the large intestine, respectively. For the case of $GLYP^{2-}$ the appropriate amount of Na^+ was randomly placed to maintain the electroneutrality of the simulating box. For each of these systems, 34 z-locations with a separation distance of 0.1 nm among them, ranging from bulk solution to the center of the lipid bilayer, were explored. Once the 34 simulations windows were completed, convergence was assessed by applying WHAM methods on 5 consecutive trajectories block of 20 ns each.

2.3. Membrane properties

Unbiased simulations were carried out along seven different systems in order to analyze the effect of the concentration of the glyphosate on the lipid bilayer properties. The first system studied was comprised of

only of 72 DPPC lipids (36 per leaflet) and SPC (Berendsen et al., 1981) water molecules, and was used as a reference. The other six systems, in addition of the lipid bilayer and water molecules, also includes different amount of herbicide molecules at a glyphosate:lipid ratio (G:L) of: (i) 1:18; (ii) 1:9 and (iii) 1:3, for each charged form of glyphosate. To this end, the appropriate number of herbicide molecules were randomly placed in the bulk water, and for the case of $GLYP^{2-}$, the necessary amount of Na^{+} was added in order to maintain the simulation box neutral. After the energy minimization process, an unbiased Molecular Dynamic simulation of 50 ns length was produced for each system. In this way, different properties of the lipid bilayer, such as mean area per lipid, thickness, deuterium order parameters, lateral diffusion coefficient, and the radial distribution function were calculated from these simulations.

3. Results and discussion

3.1. Free energy

In order to understand the difference in the behavior of herbicides in their neutral and charged forms, the excess of free energy in the presence of DPPC lipid bilayer was computed from MD simulations, and depicted in Fig. 2. In the top row of Fig. 2, the partial local mass density profile was computed for the lipid bilayer system, also indicating various lipids functional groups. In particular, we have selected phosphorus atom and oxygen 16 and 35 atoms (according of DPPC numbering in Fig. 1). In general, the excess of the free energy for transferring any of the herbicides from the bulk water to the interior of lipid bilayer ($\Delta G(z)$), depicted in Fig. 2(b), is considerably similar. It decreases as the herbicide approaches the region of the head groups, and then it increases in the hydrophobic tails area, to finally reach a peak just at the center of the lipid bilayer. However, the height of the maximum, the z-location and the depth of the minimum depend on the charge state of the herbicide. For the neutral state, the gain in the free energy is $\sim -60\,\mathrm{kJ\,mol^{-1}}$ at the location of the phosphorus atoms ($z \approx \pm 1.7\,\mathrm{nm}$). Almost no free energy change is observed when the molecule is transferred from bulk water to the center of the lipid bilayer. By contrast, the free energy minimum is $\sim -35\,\mathrm{kJ\,mol^{-1}}$ placed at the position of oxygen 16 and 35, according to Fig. 1 ($z \approx \pm 1.4\,\mathrm{nm}$)

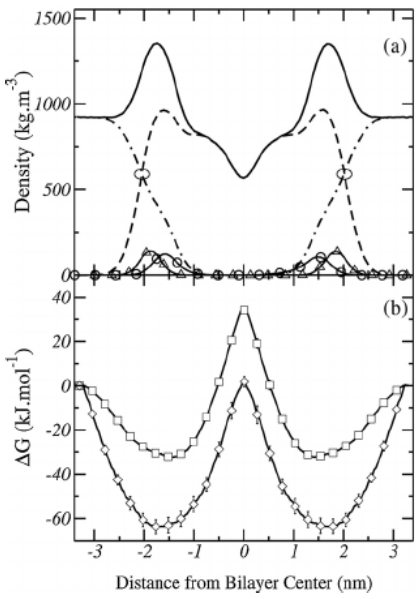

Fig. 2. (a) Partial mass density profile of the simulated system. Whole system (—); DPPC (- - -); water (— · —); phosphorus (△) and lipids' oxygen (○). (b) Transfer free energy profile for GLYP (◇) and $GLYP^{2-}$ (□). Standard error bars are included, in some cases they are the same size as the symbols.

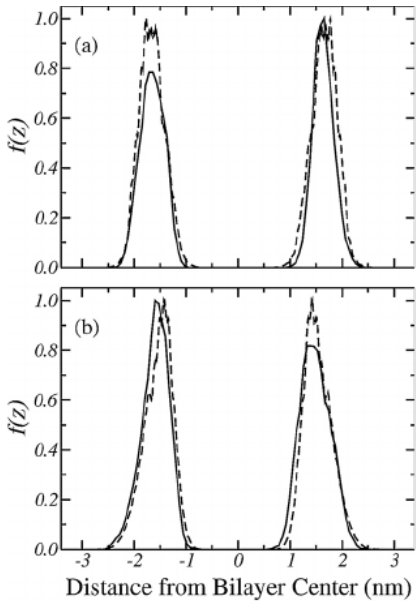

Fig. 3. Comparison of the normalized distribution function of the glyphosate as a function of z, calculated from Eq. (2) (dashes line) and the center of mass distribution of the herbicides obtained for a unbiased MD simulation (solid line). (a) Corresponds to the neutral glyphosate, and (b) to the charged form of glyphosate.

for the charged state, and the free energy barrier is $\sim 35\,\mathrm{kJ\,mol^{-1}}$ at the center of the lipid bilayer. Unfortunately, there is a lack of experimental data for the free energy of the glyphosate in the presence of DPPC. Nevertheless results showed for glycine/DPPC system, in reference (Porasso et al., 2015), in general agrees with our finding.

Fig. 3 compares the calculated distribution function, $f(z)$, from the $\Delta G(z)$, to the distribution of the mass center of glyphosate across the z-axis, which was obtained from an unbiased MD simulation. The $f(z)$ can be obtained as:

$$f(z) = \frac{e^{-\Delta G(z)/\mathrm{RT}}}{\mathscr{Z}} \tag{2}$$

where $\mathscr{Z}$ is the microcanonical partition function. For the unbiased MD simulations, we placed two molecules of herbicide, one at $z = +3\,\mathrm{nm}$ and the other one at $z = -3\,\mathrm{nm}$ (these distances are measured from the center of the lipid bilayer). Then, we released the harmonic potential, thus allowing for the free movement of the herbicides in the whole simulation box. The simulation time for this case was 50 ns. From the visual inspections of the trajectories for both unrestrained molecular dynamic simulations, it is observed that, the herbicide take only a few ps to move into the interior of the lipid bilayer, and then remain close to the head groups without escaping to the water phase along the simulation time. However, it should be noted that for the minimum of the free energy profiles ($\Delta G_{min} \sim -60\,\mathrm{kJ\,mol^{-1}}$ for GLYP and $\Delta G_{min} \sim -35\,\mathrm{kJ\,mol^{-1}}$ for $GLYP^{2-}$) results with a very low probability that any form of glyphosate could spontaneously leave the interior of the lipid bilayer. This observation also can be appreciated in Fig. 3, were the distribution functions of the mass center of the glyphosate molecule show their two maxima well-defined (at $z \sim \pm 1.5\,\mathrm{nm}$ for GLYP and $GLYP^{2+}$) and the tails of such distributions do not show that the herbicide escape to the water phase. Further, the agreement was excellent for the two systems studied, i.e. both for the location of the maximums in the probability and for the width of these distributions. The slight asymmetry that can be observed between the two maximums obtained from unbiased molecular dynamics simulations can be reduced just by increasing the statistics of the simulations.

The partition coefficient of the herbicide between the membrane

interior and the aqueous solution can be estimated from the free energy profile. By applying the minimum in the free energy, we were able to calculate the partition coefficient as:

$$K = \exp\left(-\frac{\Delta G_{\min}}{RT}\right) \quad (3)$$

considering $\Delta G_{min} = -63.6\,\mathrm{kJ\,mol^{-1}}$ for GLYP and $\Delta G_{min} = -36.7\,\mathrm{kJ\,mol^{-1}}$ for $GLYP^{2-}$. We found a partition coefficient of $\log K_{GLYP} = 9.51$ and $\log K_{GLYP^{-2}} = 4.81$. Since this organic molecule is highly soluble in water (1.01 g/100 mL at 293.15 K), and it is also a molecule with low hydrophobicity (the logarithm of the octanol–water partition coefficient is $\log K_{OW} = -4.1$), the values found for $\log K$ at both charge states of the herbicide could be considered as a specific interaction between the glyphosate and the DPPC lipid bilayer, which strongly depends on the charge state of glyphosates.

3.2. Effect of glyphosate on the membrane structure

3.2.1. Area per lipid, area compressibility and lipid bilayer thickness

The average area per lipid ($\langle A\rangle$) is related with some lipid membrane properties, such as acyl chain ordering, compressibility, and molecular packaging, among others (Anézo et al., 2003). As it been shown in a previous work (Porasso and López Cascales, 2012) the time-dependent area per lipid is also a good criterion to determine if the system has reached the steady state. The time evolution of the area per lipid, resulting of the unbiased simulations, is represented in Fig. 4 for the case of charged glyphosate (left column) and neutral glyphosate (right column) at different G:L ratios. The figures depicting the absence of herbicide, Fig. 4(a) and (e), are the same, and they have been duplicated for further clarity in comparing the different systems and concentrations. As these results suggest, all systems appear to be stable after ~5–10 ns of simulation. In Fig. 4, we also represent the average area per lipid (horizontal lines) for each system, which was calculated after discarding the first 10 ns of the simulation time (stabilization period). The calculated average area per lipid for all simulated systems are shown in Table 1. As it is observed in this Table, the $\langle A\rangle$ for the reference systems, i.e. pure DPPC, is observed to be equivalent to $0.684 \pm 0.010\,\mathrm{nm^2}$, which agree with the previous experimental results (considering that the temperature of the experiment 353 K is a bit higher than the temperature of the simulations, $\langle A\rangle_{exp} = 0.719\,\mathrm{nm^2}$ Petrache et al., 2000) and simulations data at 350 K (area per lipid values range from $0.663\,\mathrm{nm^2}$ to $0.703\,\mathrm{nm^2}$) (Porasso and López Cascales, 2012, 2009; López Cascales et al., 2012, 2014).

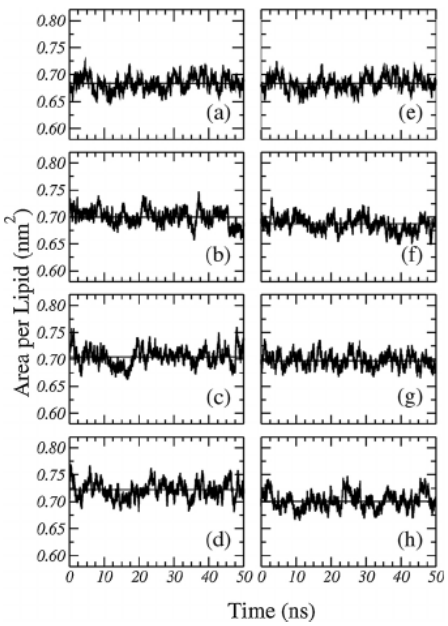

Fig. 4. Time-dependent area per lipid in presence of the $GLYP^{2-}$ (left column) and GLYP (right column) at different G:L ratios: (a) and (e) 0:72; (b) and (f) 1:18; (c) and (g) 1:9 and (d) and (h) 1:3. Horizontal solid lines represent the mean average value of the area per lipid for the last 40 ns of the simulation.

In general, there is a slight increase in the $\langle A\rangle$ with the increasing amount of herbicide. This fact is a little more noticeable for the charged form of glyphosate, although the largest increase of the area is approximately 5%, due to the presence of the herbicide.

The area compressibility modulus measures the isothermal variation of the surface tension with the membrane area, and can be calculated as (Feller and Pastor, 1999):

$$K_A = A\left(\frac{\partial\gamma}{\partial A}\right)_T = \frac{k_B T\ \langle A\rangle}{\langle \delta A^2\rangle} \quad (4)$$

were γ is the surface tension, k_B is Boltzmann's constant, T is the temperature, $\langle A\rangle$ is the average total area and $\langle \delta A^2\rangle$ is the mean square fluctuation. The resulting K_A are shown in Table 1, for the reference case the obtained value is 246 ± 5 mN/m, which agrees with the experimental value of reference (Nagle and Tristram-Nagle, 2000) at 353 K. In general, it can be appreciated that the value of K_A in presence of glyphosate, presents a decrease with the increase in the concentration of the herbicide (for both states of charge). Being more pronounced for the charged glyphosate. This behavior is consistent with the increase in the lateral diffusion coefficient (see section 3.2.3).

The thickness of the lipid bilayer was calculated as a function of the G:L ratio and the glyphosate state of charge resulting from the distance measured between the crossover points (see Fig. 2(a)) of the partial mass density values for the lipid and the solvent (Repáková et al., 2005), which is presented in Table 1. As shown in Table 1, the neutral form of glyphosate induces an increase of the lipid bilayer thickness, while the charged glyphosate produces a decrease in the thickness of the lipid bilayer. Also we observe that the change in the thickness is not linear in relation with the increasing amount of herbicide.

As for the area per lipid, the observed changes in the thickness suggest a small perturbation in the structure of the lipid bilayer due to the presence of glyphosate, which depends on the level of G:L ratios. However, higher glyphosate concentrations would lead to more significant changes in lipid structure properties.

3.2.2. Deuterium order parameter

The effect of the glyphosate on the order of the acyl chain can be studied by computing the deuterium order parameter, $|S_{CD}|$, along the lipid chain. As it has been described in previous work (López Cascales, 1996), the $|S_{CD}|$ can be calculated using the equation:

$$|S_{CD}| = \left|\left\langle \frac{3}{2}\cos^2\theta - \frac{1}{2}\right\rangle\right| \quad (5)$$

where θ is the time-dependent angle between a C–H bond along the acyl chain and the membrane plane normal (z-axis). The brackets denote the average along the simulation time and lipid molecules. In our representation of the system (united atom) θ is represented by the angle between the unitary vector perpendicular to the vector from the i to the $i+2$ CH_2 plane and the z-axis. The values of $|S_{CD}|$ range from -0.5, which corresponds to an ordering parallel to the lipid/water interphase, to 1, which corresponds to a full ordering along the normal of the lipid bilayer.

Fig. 5 depict the deuterium order parameters of DPPC in the presence of (a) GLYP and (b) $GLYP^{2-}$ at a G:L ratios of 1:18, 1:9 and 1:3; also, a pure DPPC system is included as a reference. The reference values of $|S_{CD}|$ show good agreement with the experimental values (Brown, 1982) and previous simulations (Berger et al., 1997; Petrache et al., 2000). As expected from the analysis of the mean area per lipid (Pandit et al., 2003; Porasso and López Cascales, 2009), a small decrease of the order parameter is observed for both states of charge and

Table 1
Some quantitative properties of the simulated lipid bilayer systems at different G:L ratios: $\langle A \rangle$ mean area per lipid; K_A area compressibility; D_{HH} thickness of the lipid bilayer; $\langle \theta \rangle$ mean angle between the vector joining the P–N of the DPPC lipid head group and the outward normal to the lipid bilayer; D_t^{lat} translational diffusion coefficient.

System	G:L ratio	$\langle A \rangle$ (nm^2)	K_A (mN/m)	D_{HH} (nm)	$\langle \theta \rangle$ (°)	D_t^{lat} (10^{-8}) cm^2 s^{-1}
DPPC bilayer	0:72	0.684 ± 0.010	246 ± 5	3.93 ± 0.02	90.1 ± 2.2	2.09 ± 0.20
DPPC/GLYP	1:18	0.687 ± 0.011	237 ± 4	3.97 ± 0.02	89.7 ± 2.4	3.63 ± 0.22
	1:9	0.697 ± 0.014	230 ± 4	4.00 ± 0.02	89.8 ± 2.6	5.15 ± 0.25
	1:3	0.701 ± 0.013	208 ± 4	4.07 ± 0.02	90.7 ± 2.3	21.70 ± 0.27
DPPC/GLYP^{2-}	1:18	0.700 ± 0.012	212 ± 4	3.93 ± 0.02	90.6 ± 2.5	5.40 ± 0.23
	1:9	0.704 ± 0.013	204 ± 5	3.91 ± 0.02	89.7 ± 2.7	10.40 ± 0.24
	1:3	0.722 ± 0.015	197 ± 4	3.89 ± 0.02	88.3 ± 2.8	22.20 ± 0.25

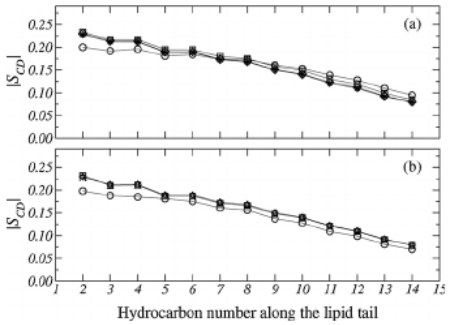

Fig. 5. The deuterium order parameter for hydrocarbon atoms in the lipid tails in presence of (a) GLYP and (b) GLYP^{2-}. Symbols for G:L ratio: (*) 0:72; (◊) 1:18; (□) 1:9 and (○) 1:3.

all G:L ratios. The maximal change correspond to the initial (C2–C5) carbon atoms, which is due to the *z*-location of the glyphosate molecules. As it has been shown in Section 3.1, the preferred location of the GLYP (for both state of charge) is near to the initial CH_2 of the acyl chains and far away from the hydrophobic regions.

3.2.3. Translational diffusion coefficient

From the MD trajectories, it is possible to calculate the displacement of the lipid molecules from the slope of the mean square displacement (MSD) curve, through the Einstein formula in two dimensions:

$$D_t^{lat} = \lim_{t\to\infty} \frac{1}{4}\frac{d}{dt}\left\langle x^2 + y^2 \right\rangle \tag{6}$$

where $\langle x^2 + y^2 \rangle$ is the displacement in the *xy* plane during the time *t*, starting at t_0, and averaging over all lipids molecules. As been described in previous work (López Cascales et al., 2006), the translational coefficient diffusion can be estimated from the slope of the MSD vs time, of the lipids, after discarding the first 10 ns were rapids movement associated with vibrations around and equilibrium point take place, rather than diffuse processes. Different studies (Camley et al., 2015; Vögele and Hummer, 2016; Venable et al., 2017) have shown that diffusion coefficient theoretical calculations are subject to different artifacts due to the scale size of the lipid bilayer, the periodic boundary conditions (PBC), and force field, which suggests that the agreement with the experimental results is not good enough. This corrections are out of the scope of the present studies, in this sense, the results shown below suggest only the tendency of the behavior of the coefficient as the concentration of herbicide increases (and the charged form of the glyphosate).

The calculated D_t^{lat} for DPPC molecules is presented in Table 1 in presence and in absence of glyphosate molecules. For the DPPC lipid bilayer, the obtained value 2.09×10^{-8} cm^2 s^{-1}, even though this value differ one order of magnitude with the experimental data (Lindblom et al., 2006), as been explained above we must consider the corrections of PBC, the scale size, and also difference in temperature at

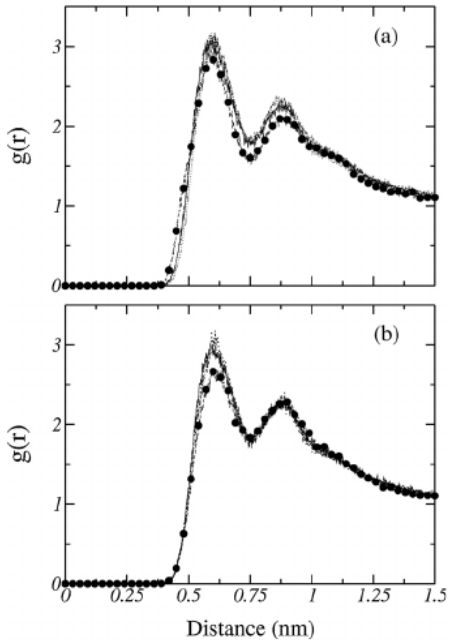

Fig. 6. Phosphorus–phosphorus radial distribution function of the DPPC in presence of: (a) GLYP and (b) GLYP − 2. At different G:L ratio: 0:72 (…… …); 1:18 (_____); 1:9 (−·− · − · −) and 1:3 (—•—).

which we performed our simulations. Furthermore, the presence of glyphosate produce a rise in the mobility of the lipid bilayer with the increasing G:L ratios, although is not linear. This effect is more significant for the charged form of the herbicide. This increase in the diffusion coefficient can be explained by considering the interaction of the herbicides with the lipid molecules, which modify the interaction between neighboring lipids. In this sense, in Fig. 6 we depict the radial distribution function between the phosphorus atoms (P atom 8 of Fig. 1) in the headgroups of the lipids. A nearest neighbor peak is clearly visible at ~0.75 nm for both charged forms at all different G:L ratios. For GLYP, the phosphorus–phosphorus radial distribution functions for all G:L ratios are similar in the first peak. However for the second peak a decrease can be clearly seen in the case of higher concentration of herbicide. For GLYP^{2-}, a notorious decrease in the first peak of the radial distribution function can be observed for the highest G:L ratio. On the one hand, behavior is consistent with the increase in the translation coefficient for the case of G:L 1:3. On the other hand, the D_t^{lat} varies just a little for the cases of lower herbicide concentration.

3.2.4. Orientational angle of DPPC head groups

To study the reordering of the lipid head group dipole in the presence of the glyphosate for both state of charge and at different G:L ratios, we compute the angle distribution of the P–N vector of the DPPC lipid molecules. The P–N vector is the unitary vector defined from the

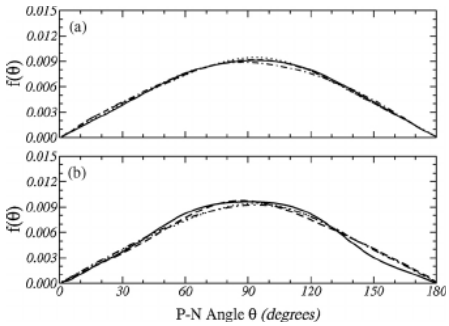

Fig. 7. Orientational distribution function of the head group of DPPC (P–N vector) with respect to the outside bilayer normal, (a) GLYP and (b) GLYP − 2. For a G:L ratio of: 0:72 (····· ···); 1:18 (– – – –); 1:9 (–·– · – · –) and 1:3 (———).

phosphorus atom (atom 8 Fig. 1) to the nitrogen atom (atom 4 Fig. 1) of the head groups of DPPC, and the angle is defined between the P–N vector and the outward normal of the membrane plane. In this sense, an angle of 0° corresponds to a P–N vector aligned with the normal vector, and an angle of 90° is parallel to the lipid-water interphase. Fig. 7 illustrates the angle distribution calculated for both leaflets after discarding the stabilization period of 10 ns, for GLYP and $GLYP^{2-}$ at different G:L ratios. It can clearly be seen that there is no observable effect on the orientation of the P–N vector due to glyphosate (neutral or charged) for all G: L ratios. This is also seen in the mean values of θ presented in Table 1, which virtually do not change due to the presence of glyphosate.

4. Conclusions

Detailed atomistic molecular dynamics simulations were carried out to study the interactions between glyphosate and a DPPC lipid bilayer in its fluid state. Two charge states of the herbicide were explored to take into account its behavior under the pH conditions in the stomach and large intestine of mammals. This study indicates that the state of charge of the herbicide influences the free energy profiles. The value and *z*-location of the minimum energy depends on the glyphosate state of charge, while the maximum is located at the center of the lipid bilayer, which is considerably higher for the charged form of the herbicide. This would indicate that the DPPC behaves as a barrier to GLYP. The main findings of the analysis of the effect of the glyphosate on the membrane properties are: the mean area per lipid is minimally influenced by the presence of the herbicides molecules, which is in line with small changes of the deuterium order parameter. But, the changes observed in the thickness of the lipid bilayer depend on both the glyphosate charge state and the G:L ratios. By contrast, the neutral state increases the thickness of the bilayer, while the charged produces a thinning of the DPPC lipid bilayer. The orientational angle distributions functions of the DPPC head groups results virtually unchanged due to the presence of glyphosate in all G:L ratios studied. Finally, the property that is most affected by the presence of glyphosate is the lateral diffusion coefficient, which is increased in one order of magnitude for the case of higher concentration of glyphosate. This effect is most noticeable for the charged state of glyphosate.

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