International Journal of Computational Bioinformatics and In Silico Modeling
Vol. 4, No. 6 (2015): 743-748
Research Article
Open Access

ISSN: 2320-0634

In silico study of glyphosate entry into lipid bilayers

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Received: 23 September 2015 Accepted: 26 October 2015 Online: 02 November 2015

ABSTRACT

The pesticide glyphosate is commonly used around the world because of its effectiveness and low persistence in the environment. Based on its solubility and ionic nature, numerous studies since the 70s have suggested that this compound should not be harmful to humans. However, recent studies have shown that it could adversely affect human health, and accumulation of glyphosate in human tissue has been correlated with teratogenic and carcinogenic effects. To affect living organisms, glyphosate must be able to cross biological membranes. In this work, we studied the ability of glyphosate to cross a membrane bilayer composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phospho-l-serine (DOPS) at different ratios. Molecular dynamics calculations were used to simulate the diffusion of glyphosate into a coarse grained model of DOPC and DOPS. The preliminary results suggested that glyphosate could cross the model membranes under certain conditions. This knowledge will be useful for assessing the impact of this herbicide on human health.

Keywords: glyphosate; phospholipid; lipid bilayer; coarse grained model; molecular dynamics.

1. INTRODUCTION

The herbicide glyphosate (*N*-(phosphonomethyl) glycine) is widely used around the world because it is highly effective at killing weeds in crops that are genetically modified to be glyphosate resistant, such as corn, soybean and rapeseed [1]. The chemical and toxicological properties of glyphosate have been widely documented [2]. Since its first use in the 1970s, several studies have concluded that glyphosate has no acute toxic effects or carcinogenicity and does not cause DNA damage [3]. However, it could cause mild oxidative stress through its effect on amino acids [4]. Overall, the mechanism of action of glyphosate is not clearly understood. New research indicates that glyphosate could have negative effects, including on soil bacteria [5-8]. Recent studies on long-term exposure to glyphosate for workers who produce or frequently apply this herbicide have shown an increased risk for cancer [9, 10]. Moreover, Guyton et al. recently detected glyphosate residues in urine and different organs and found that glyphosate may cause cancer [11]. Other studies have shown that under certain conditions glyphosate is genotoxic [12, 13] and a teratogen [14, 15], and it has also shown cytotoxic effects on different cell lines [16]. It has been proposed that glyphosate may play a role in Parkinson's disease because of its similarity to glycine [17].

When glyphosate interacts with soil, it becomes inactive rather than more bioavailable because of adsorption processes that occur within the soil [18]. However, glyphosate behaves differently in aqueous environments than in soil, and data for its behavior in this type of environment are incomplete and not entirely consistent [19]. Recent data show that various herbicides, including glyphosate, have accumulated in large quantities on the coast near agricultural settlements. Therefore, the potential risks caused by these herbicides in the marine environment need to be considered [20]. These studies, and the above new information on glyphosate toxicity, indicate that despite early conclusions on its safety, glyphosate could have a significant impact on the environment and could negatively affect human health.

In principle it can be assumed that for any substance to bioaccumulate it needs to cross one or more biological membranes. Because biological membranes are mainly composed of phospholipids, the ability of glyphosate to cross a phospholipid bilayer was investigated in the present study. Molecular dynamics (MD) simulations were used to study the interaction of glyphosate with coarse grained (CG) model membranes composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phospho-l-serine (DOPS). The coarse grained model and membrane models in particular had been reported previously [21-24]. The interaction of small molecules with the CG membranes was studied using an established method [25]. The effect of charged phospholipids on the interaction of glyphosate with the membranes was investigated by altering the membrane content of DOPC and DOPS (DOPS fraction=0–30%). The purpose of the present study was to understand how glyphosate enters membranes. The knowledge obtained could be applied to understanding the potential toxicity of glyphosate.

2. MATERIALS AND METHODS

2.1 Modeling of glyphosate

An all-atom glyphosate model was built using Visual Molecular Dynamics software (VMD)[26]. Parameterization was obtained using www.charmm-gui.org, based on ParamChem. The glyphosate molecule exhibits acid-base properties with pKa1=0.8 (first phosphonic acid), pKa2=2.3 (carboxylate), pKa3=6.0 (second phosphonic acid), and pKa4=11.0 (amine). However, the maximum efficacy of the product, as indicated by Monsanto, is at pH 5–6. Therefore, we constructed our model using pH 5–6. The obtained system was minimized for 10,000 steps using the conjugate gradient algorithm, followed by gradual heating from 0 to 310 K in 30 ps, and then maintained at a constant pressure of 1 bar. The simulation was performed using periodic boundary conditions with a time step of 2 fs, and a cut-off for non-bonding interactions that started at 10 Å and decreased until 12 Å. A simulation of 10 ns was used for geometric and energy validation of the molecular model. To assess the reliability of the proposed model, we calculated a Fourier-transform infrared spectrum for glyphosate using the IR Spectral Density Calculator Plugin, of VMD, and compared it with a literature spectrum [27]. This provided a good match and indicated the model was reliable.

2.2 Modeling of the membranes

Human cell membranes are predominantly formed from DPPC and DPPS, and in some animal tissues these compounds are also present at fractions up to 44 % [28]. In our case we adopted a simplified model for describing such cell membrane. When selecting a model, it should be as descriptive as possible of the physical world, and the computational cost should be affordable. The model proposed by Arkhipov et al. [21, 23, 29] meets both of these requirements and was adopted for the present work. The lipid bilayer was constructed using two layers of paired CG beads oriented head to tail. No pair was connected to any other pair, but the two beads within a pair were connected by a harmonic bond. The thickness of the bilayer was 25 Å, and each bead accounted for 12.5 Å of this. The two-bead CG bilayer had an area of 380×380 Å, with the area per DOPC being 70 $Å^2$. Electrostatic parameters were adopted from the work of Arkhipov et al. [22]. The system was equilibrated with a NVT time step of 100 fs at 300 K. The cut-off was identical to that seen previously (Section 2.1). Membranes were composed of various DOPC/DOPS mixtures. Four different membranes were prepared by randomly replacing some of the DOPC with DOPS. The DOPC fractions in these membranes were 70 %, 80 %, 90 % and 100 %. The DOPS fraction was not increased beyond 30 %, as this made the system unstable. All bilayer models were tested with molecular dynamics simulations of at least 500 ns. In all cases, equilibration produced energetically stable models. The data showed that the system remained geometrically stable throughout the simulation and showed the presence of roughness, which is typical of bilayer systems (Fig. 1).

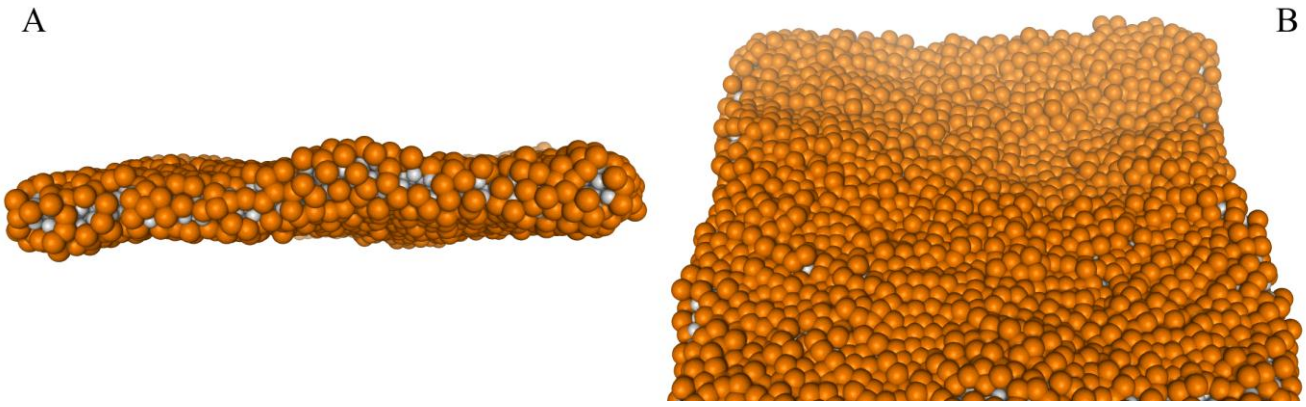

Figure 1: Cross section of the model membrane (Panel A) and top view (Panel B) after 120 ns of a molecular dynamics simulation (Panel A)

2.3 Modeling of glyphosate's interaction with the membranes

A glyphosate/membrane system was prepared for each of the four membranes. For each system, 10 replicas of the CG glyphosate model were randomly distributed on the outside of the bilayer. Each glyphosate was located at a distance of >12.5 Å from the other glyphosate molecules. Each box was designed to be large enough to contain 10 glyphosate molecules and the membrane. The final size of the box was 380×380×380 Å, and it contained approximately 10,000 molecules. MD simulations were performed using NAMD [30] software and CHARMM 27 Forcefield [31]. All systems were minimized by the conjugate gradient algorithm and gradually heated from 0 K to 300 K. The minimized structures were then balanced by coupling them to a heat bath at 300 K for 400 ps. The time step was set to 10 fs, and the rigid-bond option was used. Non-bonding interactions were treated with a cut-off value of 12 Å. A 350-ns simulation was performed for each system.

3. RESULTS AND DISCUSSION

Interaction of glyphosate molecules with the model membranes was evaluated using the distribution of glyphosate molecules along the z axis. The membrane model used for the present study completely filled the space along the *x* and *y* axes, while along the z axis it extended from −22.5 to 22.5 Å. Therefore, if a molecule of glyphosate had a *z* axis value between −22.5 and +22.5 Å, it could be assumed it was inside the membrane. The z value (17 Å, Fig. 2) for the 100 % DOPC membrane showed that most glyphosate molecules were close to the membrane surface, while a small proportion of the glyphosate molecules (z = 15 Å and 13 Å) were located deeper within the membrane. When the bilayer was composed of 90 % DOPC, a small number of glyphosate molecules were located close to the membrane surface (*z* = 12 Å), and most of the glyphosate molecules were located deeper within the membrane (*z* = 8.5 Å).

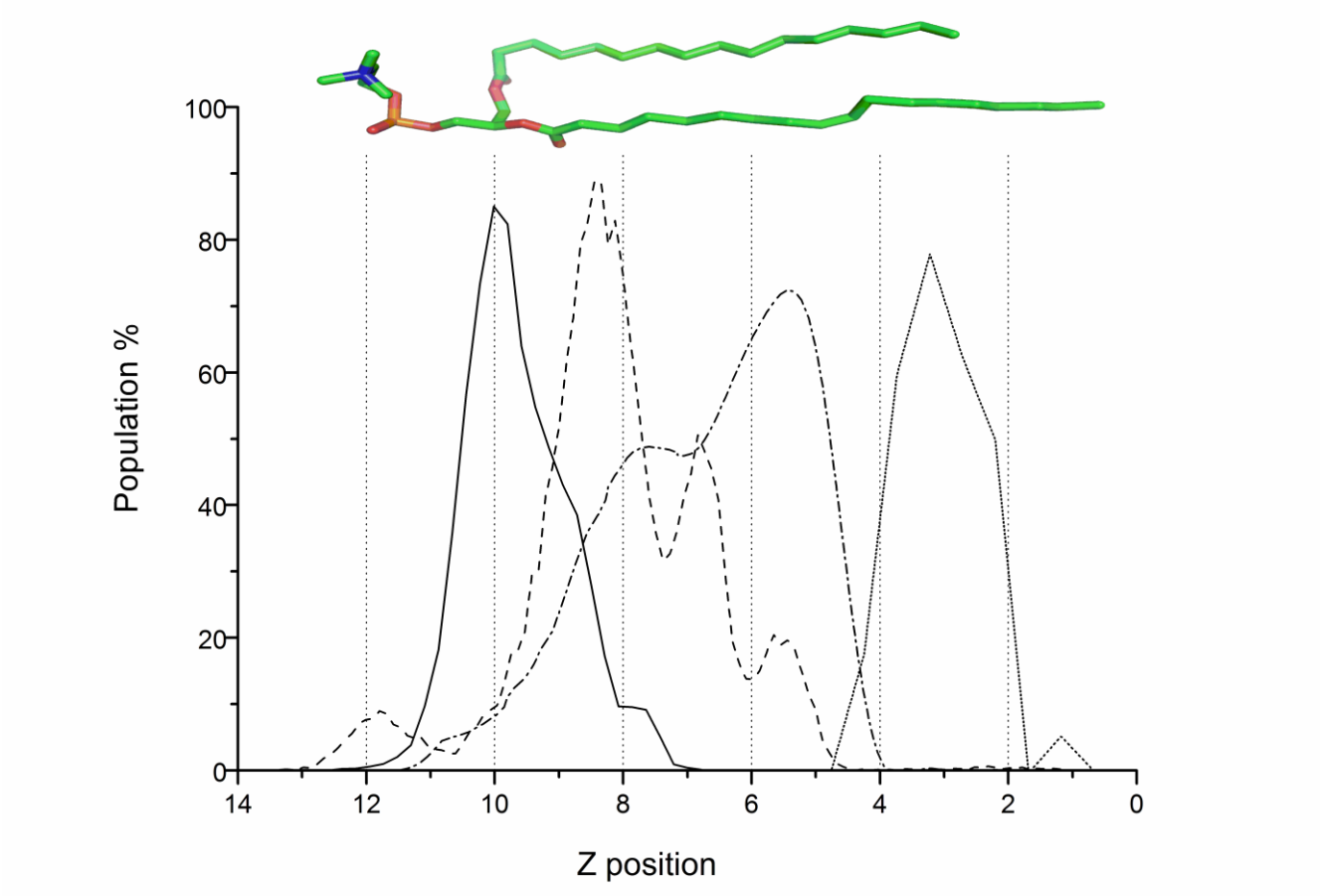

Figure 2: Distributions of the glyphosate molecules along the *z* axis for lipid bilayers with different compositions. The MDs simulation was performed at 300 K. The membrane compositions were as follows: 100 % DOPC, solid line; 90 % DOPC and 10 % DOPS, dashed line; 80 % DOPC and 20 % DOPS, dashed-dotted line; and 70 % DOPC and 30 % DOPS, dotted line.

This membrane (90 % DOPC) also showed a small number of glyphosate positioned at *z* = 7 Å and *z* = 5.5 Å. After decreasing the DOPC fraction to 80 %, more of the glyphosate molecules were located deeper within the bilayer at around *z* = 7.5 Å and *z* = 5.5 Å. With 70 % DOPC, all the glyphosate molecules were located within the membrane (*z* = 3 Å). Analysis of the trajectories for the 100 % DOPC membrane showed that all the glyphosate molecules approached the surface of the bilayer within a few nanoseconds, and then remained at the surface of the membrane for the rest of the simulation. The glyphosate initially rapidly approached the outer surface of the bilayer, and could then potentially penetrate the membrane. However, for the

100 % DOPC membrane, the glyphosate remained near the surface of the membrane where electrostatic forces prevail. Doping of the DOPC membrane with 10 % DOPS altered the behavior of the glyphosate molecules. Although phase segregation was not observed, and individual DOPS phospholipids remained uniformly distributed in the bilayer, two of the 10 glyphosate molecules behaved differently compared with their behavior in the 100 % DOPC membrane. With DOPS doping (10 %), the glyphosate molecules showed better penetration inside the bilayer (Fig. 2), with a *z* value about 4 Å less than that in the 100 % DOPC membrane. For the other two DOPS-doped membranes (80 % and 70 % DOPC), the glyphosate molecules interacted with DOPS molecules. The process for glyphosate entering the bilayer was rapid, and in all models this occurred within a couple of nanoseconds. Figure 3 shows the changes in the *z* value with time during a MD simulation for one molecule of glyphosate. Within about 20 ns, the glyphosate had moved to a *z* value of about 12 Å and then it remained at this position, at the surface of the membrane, for the remainder of the MD simulation. For the membrane containing 20 % DOPS, all of the glyphosate molecules interacted with DOPS. The glyphosate molecules were either oriented parallel to the hydrophobic tails, and were not deep within the membrane (13 Å, Fig. 2), or they were oriented perpendicular to the hydrophobic tails and were located deep within the membrane (9 Å, Fig. 2). With 30 % DOPS, the glyphosate molecules were completely embedded in the hydrophobic core and oriented perpendicular to the phospholipids.

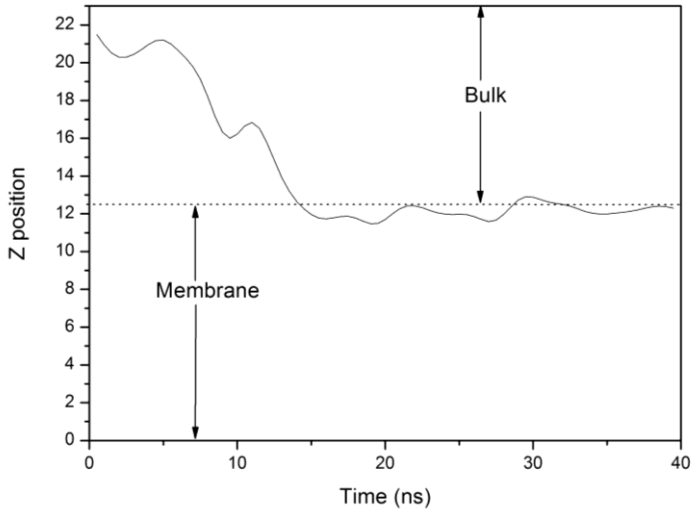

Figure 3: Changes in the position of glyphosate (solid line) relative to the membrane and the bulk solution with time during a MDs simulation for one molecule of glyphosate. The position of the herbicide is indicated by the value of the *z* coordinate.

In this case, all the glyphosate molecules were located at around 5 Å. Although glyphosate is hydrophilic and has a negative partition coefficient, the data reported here indicate that glyphosate can interact with biological membranes. In agreement with our data, Riechers et al. [32] found that glyphosate could enter vesicles in low concentrations, especially in the presence of surfactants that increase penetration. In the present study, the presence of charged phospholipids in the membrane would have the same effect as a surfactant. Moreover the glyphosate molecule according to the Lipinski rules of five [33] may be absorbed by membrane system. Entry of glyphosate into the membrane occurred even though the bilayer is made of phospholipids with hydrophilic heads on the surface and hydrophobic tails on the inside, which would be expected to repel the glyphosate and prevent it from entering the membrane.

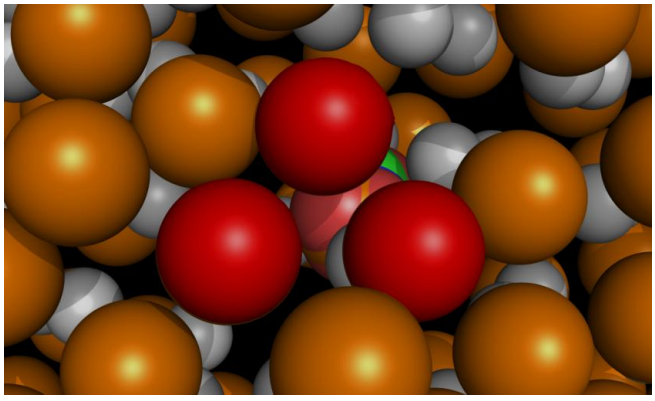

Figure 4: A molecule of glyphosate (background pink and green spheres) in close contact with three molecules of DOPS (red spheres). The orange spheres are molecules of DOPC.

To explain why glyphosate can enter the hydrophobic core of the membrane, we need to consider the composition of the membrane. In the presence of charged phospholipids, electrostatic bonds could form between the charged heads of DOPS and the herbicide. Figure 4 shows a molecule of glyphosate interacting with three molecules of DOPS. The bonds formed between DOPS and the herbicide effectively pull the glyphosate molecule into the membrane. Regarding steric effects, Fig. 5 shows a molecule of glyphosate compared with DOPC and DOPS, and highlights that glyphosate is small enough to enter the membrane without destabilizing it.

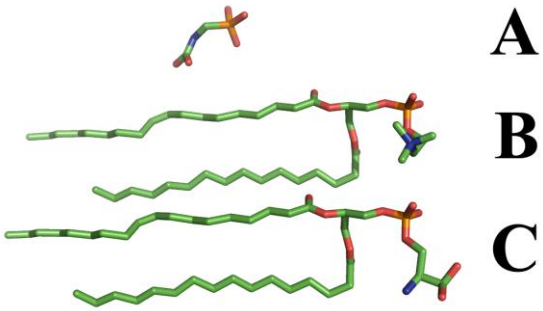

Figure 5: One molecule of glyphosate (represented as a space-filling model) (a), DOPC (b), DOPS (c). Carbon atoms are colored in green, oxygen atoms are colored in red, nitrogen atoms are colored in blue, and phosphorous atoms are colored in orange.

Glyphosate's interaction with a membrane is summarized in Fig. 6.

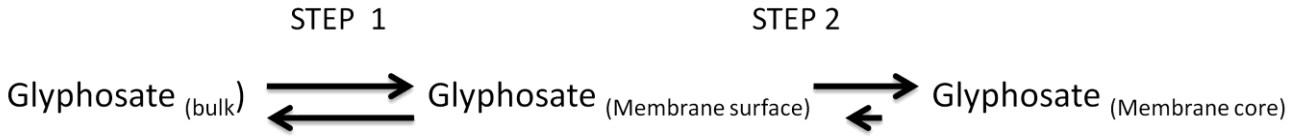

Figure 6: Schematic representation of the steps involved in glyphosate absorption into phospholipid membranes.

Initially, glyphosate molecules are attracted to the membrane surface by electrostatic interactions (Step 1), and this increases the concentration of glyphosate at the membrane surface. Then, as equilibrium with the inner core of the bilayer is perturbed, glyphosate molecules start to migrate from the surface deeper into the core of the membrane (Step 2). These results suggest that workers in direct contact with glyphosate will only absorb low concentrations of the herbicide and not experience acute poisoning symptoms. However, prolonged occupational exposure could lead to toxic effects.

4. CONCLUSION

In this study, a model of glyphosate interaction with a lipid bilayer was developed to increase understanding of real-world biological interactions of this herbicide. The mechanism of interaction of glyphosate with lipid bilayers will be affected by a number of factors, including the concentration of the herbicide and the composition of the membrane. In this study, we focused on the composition of the membrane using MD simulations, and found that glyphosate could interact with the surface of the membrane. In the presence of charged phospholipids, glyphosate penetrated into the hydrophobic core of the membrane. Therefore, the model shows that the herbicide glyphosate could negatively affect human health. This is in agreement with other literature data. The results show how glyphosate could bioaccumulate in membranes to reach toxic concentrations. Slow bioaccumulation would explain why the toxic effects of this drug have not been clearly identified, and why there are conflicting reports in the literature. This works represent an initial step in a systematic study on the toxicity of glyphosate to determine appropriate measures for safe use of this herbicide. Future research could look at improving the model developed in this study.

5. Acknowledgements

This work was supported by funding from the Ministero dell'Università della Ricerca under PRIN 2009.

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