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Study of Interaction Between PEG Carrier and Three Relevant Drug Molecules: Piroxicam, Paclitaxel, and Hematoporphyrin

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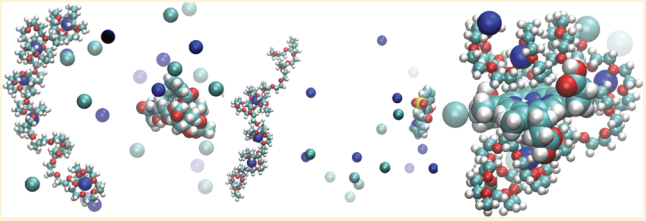
Supporting Information

ABSTRACT: Molecular dynamics simulation has been used to study the specific interactions between poly(ethylene glycol) (PEG) and three drug molecules for which PEG is used to aid delivery: paclitaxel and piroxicam, where PEG is a carrier agent, and hematoporphyrin, where PEG is covalently attached to form a "stealth shield". Simulating at physiological salt concentration, we found no evidence of any specific interaction between paclitaxel or piroxicam with PEG, but found a strong interaction for the case of hematoporphyrin. This interaction is lipophilic in nature, between the nonpolar $(CH_2)_2$ groups of the PEG and the porphin ring of the hematoporphyrin. This interaction was found to be strong enough that the PEG aggregated to the hematoporphyrin, independent of whether or not it was covalently bound. Interestingly, when the simulation was repeated in absence of salt we found evidence of this interaction being weakened. This led us to hypothesize a previously unforeseen mechanism: interaction with salt cations cause the PEG to coil around the salt ions, each ion binding to many PEG oxygens, increasing the exposure of the nonpolar ethylene groups, thus increasing the effective hydrophobicity of PEG. The Hydrophobic ethylene groups of the PEG chains adhere strongly to the hydrophobic porphin ring. Experiments involving absorption spectra measurements were conducted, and these results also indicated that presence of salt at physiological level increases the effective attractive interaction between PEG and hematoporphyrin. Taken together, our results demonstrate that while PEG, due to its solubility in both polar and nonpolar solvents, may act as a dissolution aid for paclitaxel and piroxicam, of the three drug molecules studied it will only have a protective role for the case of the hematoporphyrin.



INTRODUCTION

Poly(ethylene glycol) (PEG) has seen significant use in drug delivery. Since PEG is soluble in a wide variety of both polar and nonpolar solvents,[1] it has been widely employed as a carrier for hydrophobic drug molecules to enhance their aqueous solubility or dissolution characteristics.[2−4] Being a biologically inert polymer, PEG is not recognized by the body's defense mechanisms, and has been successfully used as a "stealth sheath", shown to inhibit uptake by the reticuloendothelial system (RES). Examples exist of the use of PEG covalently bound to proteins[5] and other drug molecules,[6] and as a carrier medium to enhance solubility, not covalently bound to the drug being delivered. In pharmaceutical nanotechnology PEG has seen use as a protective coating for both drug delivery liposomes[7] and nanoparticles.[8]

In this study we have performed a set of molecular dynamics (MD) simulations to study the interaction of PEG with three drug molecules with which it is combined in therapy: the drug molecules paclitaxel, piroxicam, and hematoporphyrin. Hematoporphyrin is used in photodynamic therapy as a photosensitizer.[9−11] For paclitaxel and piroxicam PEG is used as a carrier to enhance solubility, and is not covalently bound to the drug.[12,13] The specific PEG−drug molecule interactions are not clearly understood. Unlike these two compounds, hematoporphyrin is covalently bound to PEG, but the influence of the covalent bond on the nonbonded interactions is not understood. We have used molecular dynamics simulation with an all atom model to explore these questions. Experiments involving the application of absorption spectra measurements have been used to support the conclusions of the MD simulations.

Paclitaxel, a plant alkaloid obtained from the bark of the Pacific yew tree, is a novel antineoplastic and antimicrotubule agent.[14] It was approved for clinical use by the U.S. Food and Drug Administration in the 1990s and became a standard treatment for breast cancer, lung cancer, ovarian cancer, head and neck cancer as well as colon cancer and AIDS-related

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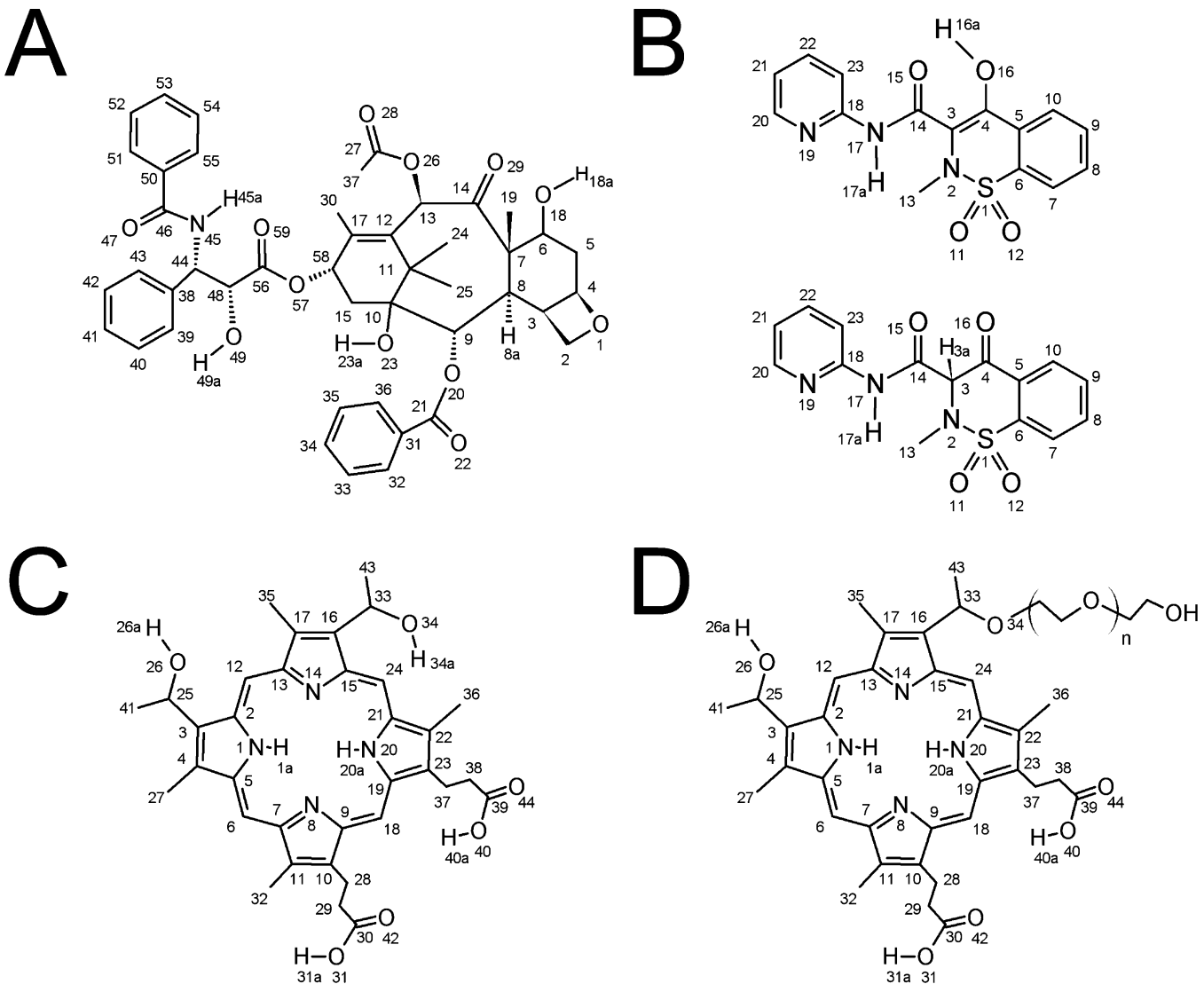

Figure 1. 2-dimensional structures of all molecules simulated (a) paclitaxel, (b) the two tautomers of piroxicam, (c) hematoporphyrin, and (d) hematoporphyrin bound to PEG.

Kaposi's sarcoma.[15] Nevertheless, the clinical utility of paclitaxel (Biopharmaceutics Classification System (BCS) Class IV drug[16]) is significantly hampered due to its low solubility and permeability. To overcome this problem, the commercial product Abraxane is formulated as protein-bound paclitaxel particles for injectable suspension. Alternatively, solid dispersions of paclitaxel in PEG/poly(lactide-*co*-glyclic acid) blends have been proposed and reported in the literature.[17]

Piroxicam is a nonsteroidal anti-inflammatory drug and used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis and rheumatoid arthritis.[18] The piroxicam molecule is a well-known example of a BCS Class II drug, with low solubility and high permeability, for which oral absorption is considered to be dissolution-rate limited. For this reason, many studies utilized piroxicam as a model drug in their efforts to develop new formulations with enhanced dissolution kinetics. Solid dispersions of piroxicam in PEG 4000[13,19] and PEG 6000[20] are examples of such formulations.

The hematoporphyrin molecule is a porphyrin obtained from hemoglobin and is used as a photosensitizer for photodiagnostic (PD) and photodynamic therapy (PDT) of malignancies.[10] It has been used in clinical cancer research as a photosensitizer, because it is readily absorbed by cancerous tissue and fluoresces effectively after excitation.[9] Jori et al.[10] studied the pharmacokinetic profile of hematoporphyrin encapsulated into small unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles in comparison to dye dissolved in phosphate buffer saline (PBS). This *in vivo* study in MS-2 fibrosarcoma-bearing mice has shown that despite a slow rate of dye accumulation in the tumor, its maximum concentration was higher than that with the PBS formulation. To improve the properties of hematoporphyrin, the porphyrin was chemically modified. Lottner et al.[21] have proposed a potential photosensitizer for PDT based on hematoporphyrin−platinum(II) complex with two PEG fragments bound to a porphyrin core. This compound exhibited a synergistic effect compared to cisplatin and hematoporphyrin alone or a combination of the drugs. Stevens et al.[22] have prepared a lipophilic derivative of hematoporphyrin by covalent attachment of stearylamine. The obtained compound was next incorporated into nanoparticles containing PEGylated lipids.

Our MD results show no evidence of any specific nonbonded interaction between paclitaxel and piroxicam with PEG polymer. Hematoporphyrin has a strong interaction to PEG,

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involving the nonpolar core of the porphin ring and the ethylene groups of PEG, that is mostly independent of whether or not the PEG is covalently bound. We found some evidence from the MD simulations that the salt concentration, through the PEG polymer forming helixes around the Na^+ cations, increases the interaction between the hydrophobic surface of hematoporphyrin when no covalent bond is present between hematoporphyrin and PEG. To test this MD result we performed measurements of UV−vis absorption spectra of hematoporphyrin dissolved in aqueous solution of PEG. These measurements showed some effect of the presence of the salt on the aggregation state of hematoporphyrin in PEG solution, in agreement with the MD results. The aim of the research presented here was to study the interaction between PEG and small hydrophobic drug molecules not easily soluble in water. Our studies should explain the role of PEG in the delivery of such drugs. Our results thus provide evidence that, while PEG may act as a dissolution aid for paclitaxel and piroxicam, it will only have a protective role for the case of the hematoporphyrin.

METHODS

Computational Methods. In this study, we performed a set of 50 ns simulations of three molecules in the presence of PEG: the drugs piroxicam, paclitaxel, and hematoporphyrin. All simulations were performed with the GROMACS 4.5.1[23,24] molecular dynamics simulation package. All molecules were simulated solvated in water at physiological salt concentration (140 mM). For the case of the hematoporphyrin we simulated both the cases of PEG covalently bound and unbound, and simulated both systems in pure water in addition to the simulation with physiological salt concentration. For piroxicam we considered, in two separate simulations, two possible tautomeres that result from the keto−enol equilibrium.[25] PEG on its own was also simulated as a control for a total of eight different systems. The chemical structure of all molecules simulated is shown in Figure 1.

For the case of hematoporphyrin, the molecule belongs to the group of "dicarboxylic porphyrins". This results from its chemical structure, which consists of a tetrapyrrole ring with two propionic acids attached at the same side of the ring (see Figure 1). The pK_a of the two acidic groups have been measured in two separate studies, with results of 5.0 and 5.4[26] and 6.0 and 6.8[27] respectively. One can thus conclude that at a physiological pH of 7.4, 76% of the Hp molecules will be deprotonated at both propionic acid groups. The protonation state can easily be altered if the local environment of the molecule is less polar, for example the environment created by the presence of PEG, and our hypothesis is that this will be the case. Since this is not a clearly resolvable issue, we have performed all hematoporphyrin simulations with the hematoporphyrin in both the neutral and di-ionic states. For the case of the piroxicam and paclitaxel, there are no basic or acidic groups present, so the assumption that these molecules will be neutral at physiological pH can safely be made.

In all systems the PEG was 45 monomer units long, a length of PEG known as PEG2000, and the size of the simulation box was 7 nm in each direction. This size was determined to be sufficiently large that finite size effects would not be an issue for the systems we have studied. All systems included approximately 11000 water molecules. Physiological salt concentration was achieved through the addition of 28 Na^+ and Cl^- ions.

While for the control sample of PEG in solution only a single system was simulated, for all other systems several separate replicas were simulated, and the results for all replicas of the same system were averaged together. Nine replicas were simulated for the paclitaxel system, and five replicas were simulated for each of the piroxicam tautomers and all eigth of the hematoporphyrin systems, with the exception of the hematoporphyrin in neutral form with covalently attached PEG at physiological salt concentration, where four replicas were simulated.

The control system of PEG in solution was started in a random PEG conformation. All systems of piroxicam and paclitaxel were started from an initial configuration achieved through ligand docking using Gold 4.0 in an unconventional way. Briefly, the conformation of the PEG was assumed to be flexible while paclitaxel, hematoporphyrin or piroxicam were considered rigid (equivalent to "protein" in classical docking). The docking program explores the flexibility using a genetic algorithm, rotatable bonds are explored with steps of 1.4 degrees. The docked structure corresponds to an optimum in the knowledge-based potential from the Goldscore scoring function. As a result, starting interaction modes where the PEG is wrapped around our studied molecules in a low-energy binding mode were obtained. A covalent bond constraint was added for hematoporphyrin. The hematoporphyrin configurations with PEG covalently attached were also started from docked configurations but all other simulations with hematoporphyrin (with the covalent bond to the PEG and/or the ions removed) were started using equilibrated configurations of hematoporphyrin with attached PEG.

The parametrization of all aforementioned molecules was made using the optimized parameters for liquid simulation all atom force field (OPLS-AA).[28] For the case of PEG this has been previously tested in our published simulation of pure PEG and PEG attached to lipid bilayers.[29] Partial atomic charges were derived in accordance with the OPLS methodology by fitting to the electrostatic potential using the RESP procedure.[30] The Merz−Kollman molecular electrostatic potential (MEP) was computed for the optimized molecule structure.[31] The structure optimization was performed at density functional theory (DFT) level using the Becke B3LYP exchange-correlation functional and the 6-31G* basis set within the Gaussian 03 program.[32] Single point calculations at Hartree−Fock level were performed with the same basis set to compute the electrostatic potential. The charge fitting to the electrostatic potential was done automatically in RESP ESP charge derived (R.E.D.) software version III.[31,33,34] Derived partial charges can be found in the molecule topologies, included in supporting materials. For ions we used the corresponding OPLS parameters and for water we used the TIP3P[35] model that is compatible with OPLS parametrization.

The simulation protocol used in this study is the same as our previous work[29,36,37] using the same force field. Periodic boundary conditions with the usual minimum image convention were used in all three directions. The LINCS algorithm[38] was used to preserve the length of covalent bonds to hydrogens. The time step was set to 2 fs and the simulations were carried out at constant pressure (1 bar) and temperature (310 K). The temperature and pressure were controlled by the Parinello−Rahman[39] and Nosé−Hoover[40,41] methods, respectively. The temperatures of the solute and solvent were controlled independently. For pressure, we used an isotropic control. The Lennard-Jones interactions were cut off at 1.0 nm, and for the electrostatic interactions we employed the particle mesh Ewald method[42] with a real space cutoff of 1.0

nm, β-spline interpolation (order of 6), and direct sum tolerance of 10^{-6}.

Experimental Methods. PEG (average molecular weight of 8000, Sigma) was dissolved in phosphate buffer pH 7.4 with or without NaCl (c_{NaCl} = 140 mM) to form two stock solutions (c_{PEG} = 100 g/L). Next these stock solution were used to prepare two sets of samples containing constant concentration of the dye ($c_{hematoporphyrin}$ = 8.3 μM) and increasing concentrations of PEG from 0 to 5.63 g/L. The sets differed in salt concentration: one set contained 140 mM of NaCl, whereas the other one was salt-free. Absorption spectra were recorded for both sets using a Varian Cary 50 Conc. UV−visible spectrophotometer.

RESULTS

Electronic Structure Calculation. To understand the possible interaction between PEG and the three studied drug molecules we employed quantum mechanical calculations, as detailed in the Methods section, to compute the electrostatic potential. The mapping of the potential on the van der Waals surface is shown in Figure 2. As expected from the structure, the porphin ring of the hematoporphyrin is characterized by a low level of charge concentration thus it is possible that the interaction with PEG can originate from hydrophobic interactions. The hematoporphyrin molecule also has two hydroxyl groups that can potentially form H-bonds with PEG oxygens and two carboxyl groups, which in charged form will not interact with PEG. For the case of paclitaxel, the situation is more complex as this molecule has a large number of polar groups, but no charged groups. Three hydroxyl groups and one amide group can potentially form H-bonds with PEG but five carbonyl and four ether oxygen atoms rather would have a repulsive interaction with PEG, thus inhibiting the formation of H-bonds, especially since these repulsive interaction sites outnumber the H-bond forming sites. For the case of piroxicam, the number of negatively charged groups which can repel PEG is also larger than the number of possible hydrogen donors. It is worth noting that large differences exist between the two piroxicam tautomers. We calculated the polar and hydrophobic surface areas, and the number of H-bond donors and acceptors using Volsurf+ (V.0.9.38),[43] and the results are shown in Table 1.

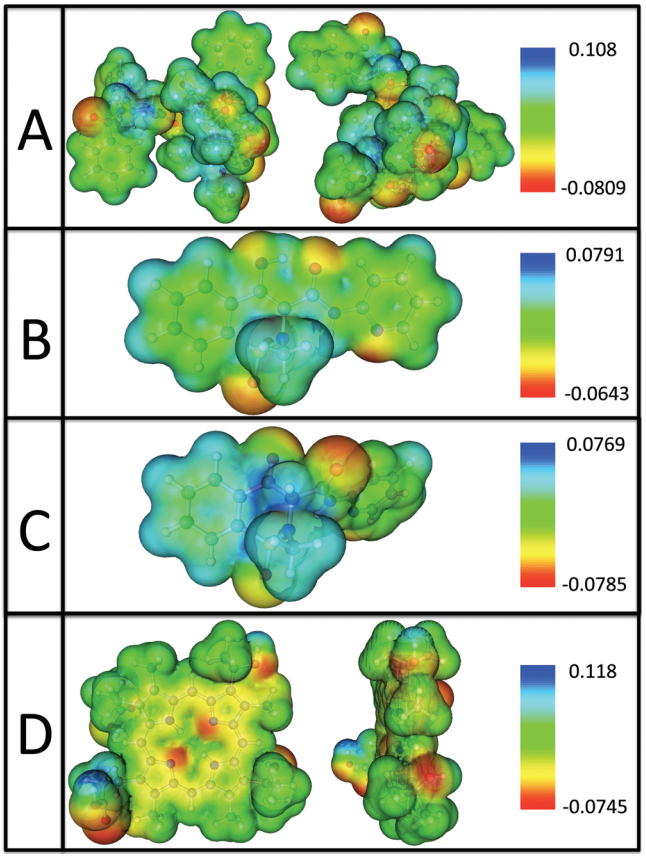

Figure 2. Electronic structure visualization (a) paclitaxel, (b) tautomer 1 of piroxicam, (c) tautomer 2 of piroxicam, and (d) hematoporphyrin.

Radius of Gyration. The radius of gyration (R_g) was calculated for the PEG polymer in all eight systems, and the results are shown in Figure 3. The *x*, *y*, and *z* components of the R_g were calculated for all systems with the system reoriented to maintain the position of the drug molecule in

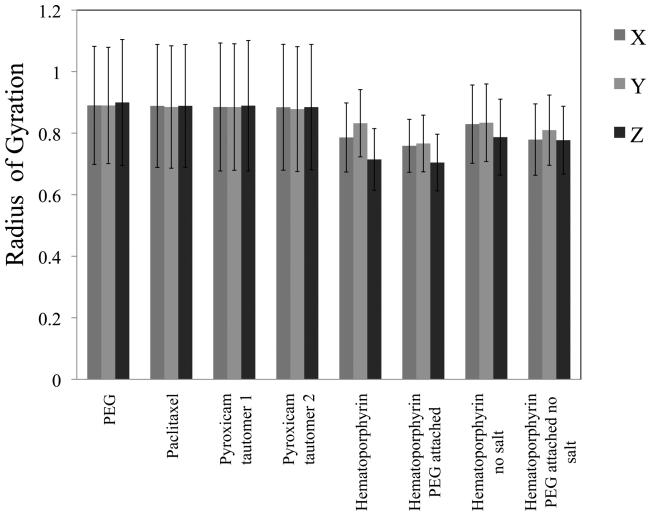

Figure 3. *x*, *y*, and *z* components of the radius of gyration for all systems simulated. For piroxicam and porphyrin, there is no change from the result for PEG in solution, indicating no interaction between PEG and the two drug molecules. for the hematoporphyrin the plane of the molecule is oriented along the *xy* plane. We see reduced radius of gyration indicating interaction between hematoporphyrin and PEG. the PEG is slightly more contracted in the axis perpendicular to the plane of the molecule (*z* axis).

Table 1. Chemical Parameters of All Molecules, Paclitaxel, both Tautomers of Piroxicam and Hematoporphyrin, Calculated Using Volsurf+

molecule	volume	mol. wt.	total SA	polar SA	hydrophobic SA	H-bond acceptor	H-bond donors
paclitaxel	1607.3	853.9	950.2	224.6	725.6	14	4
piroxicam 1	669.1	331.3	462.3	107.1	355.2	5	2
piroxicam 2	676.3	331.3	463.2	103.9	359.3	5	1
porphyrin	1291.9	624.7	821.9	115.1	706.9	6	10

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the center of the simulation box with the principle plane (plane in which the largest set of ring structures is oriented) of the molecule in the *xy* plane. We see no noticeable difference between R_g for PEG in solution and PEG with paclitaxel or either tautomer of piroxicam. An attractive nonbonded interaction would collapse the PEG around the molecule and thus reduce R_g; thus, we see evidence that there is no such interaction with PEG for the case of these two molecules. For all hematoporphyrin systems R_g is reduced, and there seems to be some anisotropy in the PEG conformation with R_g more reduced in the axis perpendicular to the plane of the molecule than parallel to it (*z* axis). For this case, the anisotropy seems greater for the two systems in the presence of ions, though this evidence is not shown consistently outside of standard deviation in all four hematoporphyrin systems.

Solvent Accessible Surface Area. We next calculated the solvent accessible surface area (SASA)[44] using VMD.[45] For each sampled configuration, we were able to use this calculation to determine the portion of the surface area of the molecule covered by PEG (the remainder being exposed to the solvent). This was achieved through calculating SASA, the total surface area of the molecule, and SASA with the PEG polymer as part of the solvent. For all systems the results over all sampled configurations were combined into a histogram, shown in Figure 4. Considering all systems with unattached PEG in physiological salt concentration (Figure 4a), we see a striking trend: paclitaxel and the two piroxicam tautomers both show a very weak and broad peak at partial PEG coverage and a sharp much stronger, dominant, peak at zero. This indicates many configurations are sampled where there is no contact at all between PEG and drug molecule. An estimate of the portion of the configurations with and without contact between PEG and drug molecule can be obtained from comparing the areas under the two peaks. We found that for the first tautomer of piroxicam 21% of configurations are unbound, while for the second tautomer the figure is 72% unbond. For paclitaxel the figure was 40% unbound. Together, this indicates evidence for lack of specific interaction between paclitaxel and piroxicam with PEG: the weak peak around partial PEG coverage is due to finite size effects; due to limited space it is impossible for the PEG polymer to avoid occasionally coming into contact with the drug molecule. The hematoporphyrin molecule, on the other hand, shows a single Gaussian (parabola in log scale depicted) peak centered around a coverage of slightly greater than 50% of the molecule surface. This indicates an interaction between PEG and hematoporphyrin. It should be noted that the meaningful result for portion of simulation time in contact with PEG is any substantial variance from 100% contact, as the simulated system is constrained by finite size effects. This results in an effective drug density in the fluid much higher than in the actual system, so any observation of the drug not in contact with the PEG for a measurable portion of the trajectory indicates and extremely weak, to nonexistent interaction.

Comparing the 4 different hematoporphyrin systems (Figure 4b,c) we see that covalent binding of the hematoporphyrin to the PEG slightly increased the portion of the molecule covered by PEG: the binding of the PEG constrains the PEG to conformations more conducive to molecule coverage. As described in the Methods section, due to the ambiguity in the ionic state of the hematoporphyrin, we performed the simulations with the hematoporphyrin in both the neutral and the di-ionic states. The possible effect of hematoporphyrin being in the di-ionic state could be a weakening of the

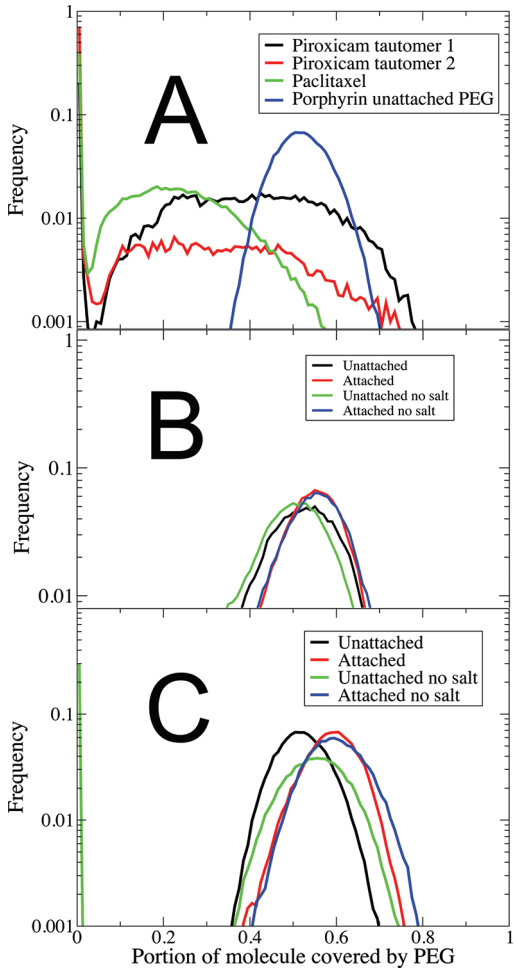

Figure 4. Histograms of the fraction of drug surface covered by PEG. comparison of (a) hematoporphyrin with both tautomers of piroxicam and paclitaxel, and hematoporphyrin with PEG covalently attached, unattached, and without salt in the solvent water, (b) in di-ionic state and (c) neutral state.

interaction between the PEG and the hematoporphyrin due to repulsion between the electronegative oxygen atoms and the negatively charged acidic groups of the hematoporphyrin. Our simulation results showed that this effect did not occur. For the neutral hematoporphyrin with the PEG unbound we see the peak at zero coverage, indicating the PEG leaving the hematoporphyrin. This provides evidence that the presence of salt could be strengthening the attractive interaction between the hematoporphyrin and the PEG when the PEG is unbound, a hypothesis we further tested using absorption spectra experiments, discussed below. All other calculated results were unchanged by the ionic state of the hematoporphyrin.

Visualization of Simulated Systems. Visualization of *freeze frames* for each of the drug molecules, performed using VMD,[45] are shown in Figure 5. As expected from previous simulations,[29,46] PEG is seen to form helices around the Na^+ cations. The PEG periodically moving completely away from the drug molecule can be seen in all systems where the peak at zero PEG coverage is present. Observing the conformations held by the trajectory of the hematoporphyrin with PEG in physiological salt concentration we see an interesting phenomenon: The wrapping of the PEG around Na^+ ions exposes the more hydrophobic CH_2 groups of the PEG on the exterior surface thus increasing the attractive interaction

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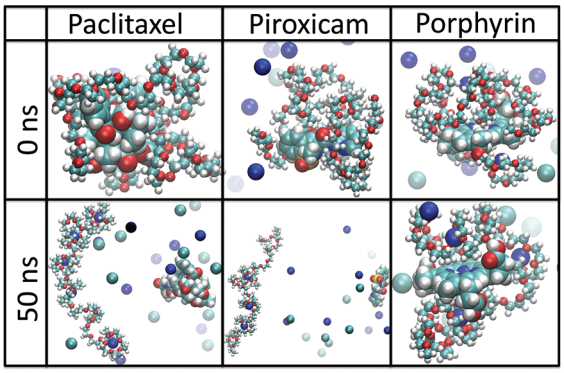

Figure 5. Images of frames from the start and end of trajectories with the three drugs, paclitaxel, piroxicam and hematoporphyrin (porphyrin). the hematoporphyrin trajectory shown is with covalently bound peg. In all three cases, the initial conformation is the structure obtained through docking.

between PEG and the hydrophobic central porphin ring of the hematoporphyrin. Thus, the salt can be seen to enhance the interaction between hematoporphyrin and PEG, a result that is supported by our observation, from our SASA analysis, that in the absence of salt the PEG was seen to occasionally drift away from the hematoporphyrin, indicating weaker interaction than in the case of salt at physiological concentration.

PEG Interaction with Ions. Since it is well established that PEG interacts strongly with cations we examined briefly its interaction with Na^+ ions. To evaluate the interactions between Na^+ ions and PEG oxygen we used a distance criterium of 0.350 nm.[47] The number of ions bonded with the whole PEG chain as well as the number of oxygen atoms coordinated by single ions are given in Table 2. As can be seen the presence of

Table 2. Number of Ions Bonded with PEG Chain, Number of PEG Oxygen Atoms in Ion Coordination Shell

molecule	bonded ions	oxygens
pure PEG	2.5 ± 0.4	5.9 ± 0.3
paclitaxel	2.4 ± 0.3	5.9 ± 0.1
piroxicam tautomer 1	2.2 ± 0.3	5.8 ± 0.2
piroxicam tautomer 2	2.3 ± 0.3	5.8 ± 0.2
hematoporphyrin	1.9 ± 0.2	6.0 ± 0.2
hematoporphyrin PEG attached	1.8 ± 0.3	6.0 ± 0.2

paclitaxel and piroxicam does not affect the interaction between PEG and ions as the number of bonded ions is the same as for the case of PEG simulated without drugs. For the case of hematoporphyrin, the number of bonded ions is slightly decreased. This probably results from the fact that a substantial part of the PEG chain is involved in the interaction with hematoporphyrin.

Absorption Spectra Experiments. We next measured the absorption spectra of hematoporphyrin in varying PEG concentrations, in order to find experimental evidence of the effect of presence of salt (NaCl) in solution on the interaction of PEG with hematoporphyrin. Figure 6a shows the absorption spectra of aqueous solutions of hematoporphyrin containing 140 mM NaCl and a range of concentrations of PEG. As can be seen hematoporphyrin molecules are strongly aggregated in aqueous solution. It is well-known that porphyrins form dimers or higher aggregates after dissolution in aqueous environment.[48,49] The addition of PEG solution results in a gradual reduction of the aggregation, which was manifested as an increase of intensity of the band at 394.5 nm and decrease of intensity in the shoulder centered at 375 nm. Similar changes were observed for the case of the aqueous solution of hematoporphyrin in the absence of NaCl.

The ratio of absorbance at 394.5 to 375 nm ($A(394.5)/A(375)$) can be applied as a rough estimator of the aggregation phenomenon in the hematoporphyrin solution. These ratios were calculated for two sets of spectra (with or without NaCl) and are presented in Figure 6b. One can notice an effect of the presence of the salt in the solution on hematoporphyrin aggregation. At low concentration of PEG, the introduction of NaCl caused a decrease in the ratio of $A(394.5)/A(375)$ in comparison to the value in solution without NaCl. The Na^+ cations present in solution promote the formation of hematoporphyrin dimers. When we increase the concentration of PEG above 1 g/L, however, the value of $A(394.5)/A(375)$ increases, thus the degree of aggregation slightly decreases. This can be explained through the mechanism we found in our MD simulation that results in complexation with cations increasing the effective hydrophobicity of the PEG. It is well-known that hematoporphyrin dissolved in organic solvents are present in monomeric form. As an illustration of this we present in Figure 6a an absorption spectrum of hematoporphyrin dissolved in dimethylformamide (DMF, blue line).

■ DISCUSSION AND CONCLUSIONS

Our MD simulation study has shown a clear result: there is no attractive non bonded interaction between piroxicam and paclitaxel with its PEG carrier molecule, however due to the hydrophobic center of the porphin ring structure there is a clear attractive interaction between PEG and hematoporphyrin. While PEG is known as a hydrophilic polymer, it is in fact soluble in both polar and nonpolar solvents,[1] thus in solution one would expect a stronger interaction between PEG and more hydrophobic molecules. Hydrophobicity of a given compound can be quantified using so-called lipophilicity parameters. A partition coefficient between an organic solvent phase and water, P, is one of the coefficients that could be used to describe an affinity of the compound to the hydrophobic environment.[50] This lipophilicity parameter is conveniently defined as the ratio of concentrations of the compound, as the neutral molecule, in n-octanol and aqueous phases. This coefficient is used in the logarithmic form and labeled log P. Since for hematoporphyrin log P has been measured to be 4.69,[50] for piroxicam log P values of 1.6,[51] 1.98,[52] and 3.0[53] have been obtained, and for paclitaxel 3.5,[54] it is not unexpected that the interaction between hematoporphyrin and PEG is stronger than that between PEG and piroxicam and paclitaxel; PEG is not completely hydrophilic and a more hydrophobic molecule will thus prefer to be in contact with PEG than with water. Also, for the case of paclitaxel and piroxicam a specific interaction between drug and PEG in the blood plasma is not expected; PEG is used to enhance solubility in the intestine during solubilization, PEG on its own is very poorly absorbed. We calculated the polar and hydrophobic surface areas for all three molecules and the ratio of these (polar/hydrophobic) was found to be roughly what would be expected from the log P values: for both piroxicam and paclitaxel 0.3, and for the hematoporphyrin the smaller value of 0.16. It should be added that hydrophobic surface area alone is not the sole factor driving the stronger interaction between the hematoporphyrin and PEG. The hematoporphyrin surface is flat, unlike the

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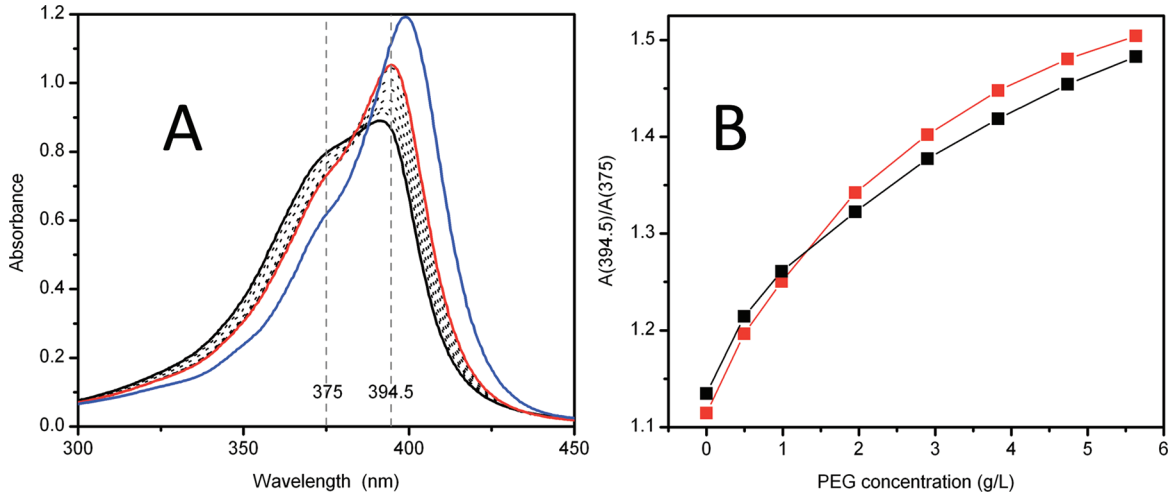

Figure 6. (a) Absorption spectra of hematoporphyrin in DMF (blue line) and in varying PEG concentrations (from 0 g/L (bold black line) to 5.63 g/L (bold red line)) in the presence of NaCl (c_{NaCl} = 140 mM). (b) Dependence of the ratio of absorbance at 394.5 to 375 nm (A(394.5)/A(375)) on the concentration of PEG in aqueous solution with NaCl (red squares) and without NaCl (black squares).

hydrophobic surfaces of paclitaxel and piroxicam, thus entropically favoring this interaction.

Concerning hematoporphyrin, we see evidence that this interaction is enhanced by the presence of salt, even at the relatively low physiological concentration, but only for the case where there is no covalent bond between PEG and hematoporphyrin. This indicates that, when bound, the PEG may be oriented by the bond into a configuration favorable to nonbonded interactions; when not bonded the effect of the salt, preferentially configuring the hydrophobic surfaces of the PEG to be exposed, is more pronounced.

The aggregation phenomenon of hematoporphyrin in an aqueous environment was previously studied and the dimerization equilibrium constant, K_D, for hematoporphyrin at neutral pH and 37 °C were found to be 2.8 × 10^5 M^{-1}.[55] Thus, hematoporphyrin molecules in aqueous solutions are present mostly in the aggregated form. Since both the interactions between PEG and hematoporphyrin, and hematoporphyrin dimerization are driven by contact with the hydrophobic surfaces of hematoporphyrin, it can be assumed that these interactions are in competition. Thus, increased interaction with PEG will result in the observation of a decreased level of dimerization.

The formation of hematoporphyrin dimers or higher aggregates affects the absorption spectra, causing a decrease in the $A(394.5)/A(375)$ ratio and a broadening of the absorption bands, as can be seen in Figure 6a. Therefore, the shape of the absorption bands and the value of the $A(394.5)/A(375)$ ratio can provide information on the aggregation between molecules. In the absence of PEG the equilibrium is strongly shifted toward the aggregated forms of porphyrins due to the high value of K_D. The introduction of PEG to hematoporphyrin solution resulted in the formation of complexes between the hematoporphyrin molecules and PEG chains. As a consequence, the amount of hematoporphyrin molecules being in monomeric form increased, manifested by changes in the UV−vis spectra and the rise of the $A(394.5)/A(375)$ ratio (black line in Figure 6b). Moreover, the presence of the Na^+ cations in the system caused a greater increase of the value of this ratio. This fact can be explained by the complexation of Na^+ cations by PEG chains, which resulted in the exposure of ethylene groups on the exterior of the resultant PEG loops around the cations. Thus, the PEG chains became more effectively hydrophobic and could as a result form more stable complexes with hematoporphyrin.

In summary, we see evidence of the presence of a specific attractive interaction between PEG and drug, that is strengthened by a physiological level of salt, for hematoporphyrin, but not for paclitaxel or piroxicam.With both piroxicam and paclitaxel PEG is used primarily as a dissolution agent to increase uptake in the gut. This action results from the fact that PEG is soluble in both polar and nonpolar solvents, thus a drug molecule that is normally too hydrophobic to enter the bloodstream can be helped to enter through the presence of PEG; the hydrophobic molecule dissolves in PEG and the PEG dissolves in the bloodstream. PEG is, however, able to play an additional role of protection from the bodies defense mechanisms, as it does in PEGylated liposomes. In order for PEG to play this role, a specific attractive interaction is required in so that PEG will surround individual drug molecules. We see evidence that such a role can be played by PEG for the hematoporphyrin but not for the paclitaxel or piroxicam.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Topologies (.itp files) and structures (.gro files) of the three drug molecules and PEG built in this study are included as a zip file. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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