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Biophysical Studies in Polymer Therapeutics: The Interactions of Anionic and Cationic PAMAM Dendrimers with Lipid Monolayers

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Biophysical Studies in Polymer Therapeutics: The Interactions of anionic and cationic PAMAM Dendrimers with Lipid Monolayers

Understanding how polymers interact with biological membranes is important for the development of polymer based therapeutics and wider biomedical applications. Here, biophysical methods (surface pressure measurements, external reflection FTIR) have been used to investigate the interaction between PAMAM dendrimers (Generation 5 or 4.5) and anionic (DPPG) or zwitterionic (DPPC) model membranes. We observed a concentration-dependent binding behaviour of both PAMAM species to both model membranes; however, equivalent levels of penetration into DPPC monolayers required approximately 10-fold higher dendrimer concentrations than for penetration into DPPG monolayers. Overall, the anionic PAMAM G4.5 showed a slightly better penetration ability which could be caused by repulsive forces towards the lipid layers. In comparison, increasing concentration of cationic PAMAM G5 leads to saturation of adsorption at the anionic lipid surface before penetration into the lipid layer likely driven by electrostatic attraction. Our studies also showed that physiologically relevant concentrations of sodium chloride (144 mM) decreased PAMAM penetration into DPPG monolayers but did not significantly affect the dendrimer-DPPC interaction. These results provide an insight into the mechanism of interaction between charged dendritic polymers with a lipid interface and show that the nature of such interactions are affected by lipid headgroup, dendrimer charge and solution salinity.

Keywords: PAMAM dendrimers; anionic; lipid monolayer; surface pressure; FTIR

Introduction

Poly (amidoamine) (PAMAM) dendrimers are monodispersed polymeric systems characterised by a well-defined molecular architecture [1, 2]. In the context of polymer therapeutics, PAMAM dendrimers have been explored for multiple purposes: as drug/gene carriers [3, 4, 5] and as biologically active polymers *per se*. In particular, full generation PAMAM dendrimers have been investigated for use in gene delivery [6] as the positive charges conferred by the primary amino groups on the surface of the dendrimer make them ideal for complexing with negatively charged DNA [7, 8].

While PAMAM dendrimers have proved to be efficient gene transfer agents, their transition into medical products is somewhat limited by their toxicity, previously reported to be concentration-, generation-, and charge-dependent [9, 10, 11]. Therefore, studies have investigated PAMAM dendrimer - cell membrane (or model membrane) interactions, to determine: (a) the mechanisms of gene transfer [7, 12, 13], (b) the mechanism of toxicity [14, 15, 16], and, most importantly, (c) physico-chemical factors affecting PAMAM toxicity [17, 18, 19], in view of tailoring the chemical structure to produce more optimised systems. Moreover, PAMAM dendrimers have displayed antibacterial properties [20, 21, 22, 23], which makes them potentially interesting candidates as drugs, *per se*.

Biophysical and *in vitro* biological studies have been carried out to elucidate PAMAM-membrane interactions, primarily using cells [24, 25], vesicles [26, 27, 28], and lipid bilayers [27, 29, 30, 31]. For studies investigating the impact of lipid composition and solution properties on polymer binding to model membranes, lipid monolayers at the air/water interface are well-established physico-chemical models. Lipid monolayers have been used extensively to investigate lipid layer phase states [32], protein/peptide binding to lipid surfaces [33, 34], and DNA adsorption [35, 36]. Their simplicity does not reflect the complexity of biological scenarios, but in turn, it does allow an excellent control over individual features and, as a result, it allows assessment of the individual contribution of different factors. In contrast to other model systems, lipid monolayers allow the lipid composition and lipid density to be selected and modified without restriction. Lipid monolayers have proved to be good models to understand interactions of antimicrobial peptides [37, 38] with bacterial membranes. In general, results obtained from monolayer-based experiments are in accordance with observations on other membrane models [29, 37, 39].

Herein, we present a systematic study looking at PAMAM dendrimers in lipid monolayers as a simple and tuneable model system to assess polymer – biological membrane interactions. We have investigated the impact of polymer charge and concentration on its interactions with a zwitterionic and an anionic model membrane. Specifically, to produce the model membranes two saturated phospholipids were selected with differently charged head groups; 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, zwitterionic) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DPPG, anionic) (Figure 1), which are predominant polar lipids in eukaryotic and prokaryotic membranes, respectively [40, 41]. Due to their simplistic structure, they are well-established model lipids to study membrane interactions under a wide range of conditions. As unsaturated phospholipids tend to create disorder in the hydrophobic region and hinder formation of the condensed phase in which the interaction experiments are to be carried out, the saturated 1,2-dipalmitoyl-phospholipids have been chosen for this study.

Surface pressure measurements were employed to determine penetration of polymers (details in Table 1, Figure 1) into lipid monolayer surfaces and to assess polymer selectivity towards different lipids. To measure the impact of polymer concentration and charge, studies were carried out on PAMAM dendrimers generation 5 (G5, cationic) and generation 4.5 (G4.5, anionic). Both dendrimers carrying 128 functional surface groups and are of a similar size. Linear polyethylene glycol (PEG) of a similar size (20,000 g mol^{-1}) was used as a negative control; as a model of a non-ionic, non-toxic polymer. Further, as most PEGylated products on the market contain linear PEG, it was decided that linear PEG would be a clinically relevant comparator. FTIR studies were carried out to complement and clarify the findings from surface pressure measurements.

Experimental

Materials

Lipids were obtained from Avanti Polar Lipids, Inc (AL, USA). The solutions of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; synthetic purity >99%, 734 g mol^{-1}) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DPPG; synthetic purity >99%, 745 g mol^{-1}) were prepared in chloroform (Sigma Aldrich UK, 99%+) to a concentration of 0.5 mg mL^{-1}. PAMAM G5 (~28,800 g mol^{-1}, ethylenediamine core), PAMAM G4.5 (~26,300 g mol^{-1}, ethylenediamine core) and Polyethylene glycol (PEG, 20,000 g mol^{-1}), the phosphate salts and solvents were obtained from Sigma Aldrich (Dorset, UK). Sodium chloride was obtained from Fisher Scientific UK Ltd (Loughborough, UK). Aqueous Phosphate buffer solution (20 mM) at pH 7 was prepared in house with UHQ water at 18.2 mΩ (ELGA purelab). Enriched salt buffer was prepared adding 144 mM sodium chloride to phosphate buffer pH 7.

Surface Pressure Measurements

Experiments were performed on a Langmuir-Blodgett trough (model 611, Nima Technology Ltd, Coventry, UK) equipped with barriers used for monolayer compression. Lipid monolayers were created at the air/liquid interface and surface pressure measurements were carried out using the Wilhelmy plate method as previously described [34, 38]. The trough was filled with 80 mL of phosphate buffer, onto which 25 μL of a 0.5 mg mL^{-1} lipid solution was spread on the surface. After allowing up to 15 min for the chloroform to evaporate, surface pressure versus area per molecule isotherms were recorded; and the lipid layer was compressed and held in the condensed phase at 21 ± 2 mN m^{-1}. Two millilitres of concentrated polymer solution (0.1 - 25 mg mL^{-1} in phosphate buffer) were then introduced into the buffer subphase with a custom-made needle to obtain concentrations ranges from 0.0024 – 0.6 mg mL^{-1}. The polymer-lipid interaction was recorded as a function of surface pressure versus time until equilibration and the maximum change in surface pressure determined.

External Reflection FTIR Spectroscopy

ER-FTIR spectra were recorded using a ThermoNicolet Nexus instrument (Madison, WI, USA) fitted with a monolayer/grazing angle accessory (Specac 19650 series, Kent, UK). The instrument was also fitted with a mercury cadmium telluride (MCT) detector and connected to an air dryer to purge the instrument of water and carbon dioxide. A small PTFE trough was used to control lipid compression and allow studies at the air/water interface to be carried out. The grazing angle accessory was set to a grazing incident angle of 55° from the surface of the trough. Polymer-lipid interactions were analysed using external reflectance as described by Lad et al [38]. All FTIR spectra were collected at a resolution 4 cm-1 where 256 interferograms were collected, co-added and ratioed against a background spectrum of D_2O buffer solution. In each experiment, 9.5 mL of 20 mM sodium phosphate buffer (pD 7.0) were placed in the trough and a background single beam spectra recorded. The lipid solution (5 µL of 0.5 mg mL^{-1}) was spread onto the surface of the buffer and compressed to 21 ± 2 mN m^{-1} predefined using surface pressure measurements, as described previously [38]. After collection of the lipid spectra, 500 µl of PAMAM solution was introduced into the phosphate buffer subphase in stages to allow for measurements of binding at different polymer concentrations of 0.0024, 0.0125, 0.018 and 0.024 mg mL^{-1} for binding to a DPPG monolayer and 0.02, 0.06, 0.12 and 0.3 mg mL^{-1} for binding to DPPC monolayer. These different ranges of concentrations were selected based on the concentrations that had shown a similar response (similar Δ surface pressure) in the previous experiments (figure 3). Spectra were collected continually for the first 30 min after polymer injection, and one spectrum every 15 min for a total of 2 h until the next polymer addition. The interaction of the polymer with the lipid monolayer was observed by monitoring the amide region, 1,700 – 1,550 cm^{-1}, and the CH_2 asymmetric and symmetric stretching frequencies (2,850 – 2,930 cm^{-1}). To correct for any water vapor present, H_2O and HOD spectra were scaled and subtracted against dendrimer adsorbed spectra, the degree of subtraction was dependent on the adsorption time as well as the amount of H/D exchange. No further processing was performed.

Results

Effect of polymer charge on polymer-lipid interactions

In this initial study, we selected two PAMAM dendrimers (G4.5 and G5) and PEG. The polymers had very similar molecular weights (approx. 30,000 Da, see Table 1), but differed in charge. While at neutral pH the full-generation PAMAM dendrimers (including G5) have the primary amines of their surface groups protonated and therefore carry a cationic charge [42, 43], the carboxylate surface groups of their half-generation counterparts (e.g. G4.5) are deprotonated and account for the anionic charge of these dendrimers. Considering the charge difference of the otherwise structurally similar PAMAM dendrimer molecules, we studied their interaction with differently charged lipid monolayers and compared the results to those of linear, non-charged PEG 20,000. Aqueous solutions of these three polymers were exposed to DPPC and DPPG monolayer, as mimics of eukaryotic and procaryotic membranes. Surface pressure changes over time were recorded for PAMAM G5 and G4.5 and for PEG (all at 0.061

mg mL^{-1}) interacting with DPPC and DPPG monolayers compressed to the condensed phase (Figure 2). This technique records changes in surface pressure, indicative of a penetration of the molecules into the lipid monolayer. The addition of both PAMAM dendrimer types at 0.061 mg mL^{-1} produced distinctively different interaction profiles for DPPG suggesting a different interaction mechanism and showed similar profiles with significantly less interaction for DPPC monolayers.

More specifically, PAMAM G5 interaction with DPPG monolayers resulted in a fast increase reaching its maximum surface pressure within 10 min and remaining in equilibrium at this level; whereas in comparison PAMAM G4.5 first induced a small decrease in surface pressure prior to a slow but steady increase reaching its maximum surface pressure within 60 – 120 minutes of the observation period. The interaction of both dendrimers with DPPG monolayers resulted in a similar magnitude of change in surface pressure (4.32 ± 0.33 mN m^{-1} and 5.69 ± 0.58 mN m^{-1} PAMAM G5 and PAMAM G4.5 respectively, Fig. 2d), which indicates that both dendrimers were able to penetrate the DPPG monolayer to a similar extent.

Conversely, little to no penetration of PAMAM dendrimers into the zwitterionic DPPC monolayer occurred at the dendrimer concentration of 0.061 mg mL^{-1} (Figure 2b and c, open symbols). Only minor increases in surface pressure were observed for dendrimer interaction with DPPC; 0.84 ± 0.08 mN m^{-1} and 1.34 ± 0.25 mN m^{-1} for PAMAM G5 and PAMAM G4.5 respectively (Figure 2d). This was probably because of the net-neutral charge of the DPPC headgroups did not offer sufficient electrostatic attraction to facilitate adsorption and penetration of the PAMAM dendrimers at this concentration.

In comparison, the non-charged PEG 20,000 displayed a different behaviour, showing no significant increase in surface pressure for interaction with either DPPG or

DPPC (Figure 2a), indicating that the polymer was unable to penetrate either monolayer. The interaction profiles showed a small increase in surface pressure (< 1 mN m^{-1}) relating to the injection process within the first two minutes and then the surface pressure remained in equilibrium. The lack of interaction of PEG was somewhat expected as this polymer, a clinically relevant comparator, is well known for its biocompatibility.

Effect of polymer concentration on polymer-lipid interactions

Surface Pressure Measurements

Upon observing a difference in PAMAM dendrimer binding to anionic DPPG and zwitterionic DPPC, the effect of polymer concentration on penetration into the lipid layers was investigated using surface pressure measurements. The changes in surface pressure with increasing polymer concentration are compared in Figure 3; for PAMAM G5 (3a), PAMAM G4.5 (3b) and PEG 20,000 (3c) interacting with DPPG (filled symbols) and DPPC (open symbols) monolayers respectively.

Both PAMAM species showed a concentration-dependent surface pressure increase related to greater penetration when more dendrimer molecules were present in the phosphate buffer subphase. Furthermore, the interaction profiles with the lipid monolayers described above for the PAMAM concentration of 0.06 mg mL^{-1} were observed over the whole concentration range studied.

The concentration-dependent changes in surface pressure for PAMAM G5 are displayed in Figure 3a. For the interaction with DPPG, the surface pressure change observed increased from approximately 1.7 to 7.6 mN m^{-1} at concentrations of 0.0024 to 0.3 mg mL^{-1}. Interestingly, for concentrations above 0.18 mg mL^{-1}, penetration into DPPC monolayers was also observed (which had not been detected at lower concentrations), inducing a maximum change in surface pressure of approximately 3.1 mN m^{-1} for the highest concentration of 0.3 mg mL^{-1}.

The results of the study of PAMAM G4.5 are depicted in Figure 3b. The surface pressure change observed for interaction with DPPG increased from approximately 0.8 to 5.7 mN m^{-1} at concentrations of 0.012 to 0.06 mg mL^{-1}. Furthermore, similarly to what observed for PAMAM G5, the surface pressure change observed for interaction with DPPC monolayers increased with higher concentrations (from approximately 1.3 to 4.0 mN m^{-1} for concentrations from 0.06 – 0.3 mg mL^{-1}) indicating dendrimer penetration at all concentrations studied.

Interaction of PEG 20,000 with DPPG and DPPC monolayers was investigated over a wide concentration range (0.0024 – 0.6 mg mL^{-1}) (Figure 3c) and the interaction profile described earlier (i.e. no penetration) remained the same for all concentrations studied. This observation could be explained with PEG being a non-charged polymer and therefore not involved in electrostatic interactions.

External reflection FTIR spectroscopy

As surface pressure measurements are sensitive to polymer penetration into the lipid layer, but not total adsorbed amount, external reflection FTIR spectroscopy was used to investigate adsorption of PAMAM G5 to the lipid interface. This method provided a measure of total mass of polymer at the lipid/water interface through measurement of the peak area around the Amide I peak (1,700-1,550 cm^{-1}) associated with the peptide bond.

Figure 4 shows Amide I peak area plotted against polymer concentration for PAMAM G5 binding to DPPC and DPPG monolayers. The presence of an increasing peak area around the Amide I peak confirms the presence of PAMAM G5 at the lipid interface. However, PAMAM G5 adsorption to the different lipid surfaces varies considerably. A visible amount of dendrimer was adsorbing at very low concentrations (0.0024 mg mL^{-1}) with an observed equilibrium peak area starting at 0.05 for binding to DPPG. In comparison, when the interaction with DPPC was investigated less dendrimer adsorption was observed with a peak area maximum of 0.015. The peak area maximum for DPPC occurred at a concentration that was a magnitude larger than the maximum peak area observed for DPPG.

For PAMAM G5 adsorption to DPPG, the lipid surface appeared to become saturated by the dendrimer towards the maximum concentration tested (peak area at 0.0125 mg mL^{-1} equalled 83% of that observed for 0.025 mg mL^{-1}). In contrast, measurements of penetration by surface pressure showed no evidence of saturation even at much higher concentrations of PAMAM G5 (maximum concentration 0.3 mg mL^{-1}). The comparison of results from the two techniques suggests that dendrimer accumulation at the surface of the lipid occurs before penetration into the monolayer.

Effect of sodium chloride in the buffer subphase on polymer-lipid interactions

Sodium chloride is an important component of biological systems. Concentrations of sodium chloride observed in biological systems are different in different cellular environments. As PAMAM dendrimers carry charges, their interaction with the lipid monolayer are likely to be influenced by these charges, and, as such, by the presence of sodium chloride. We studied PAMAM interaction with DPPG and DPPC monolayers in presence of sodium chloride (144 mM) in the phosphate buffer subphase. The interactions were studied at a PAMAM concentration of 0.06 mg mL^{-1} for DPPG and 0.3 mg mL^{-1} for DPPC; these concentrations selected as they corresponded to equivalent surface pressure changes without addition of salt (see Figure 3). The results are summarised in Table 2.

The sodium chloride addition to the phosphate buffer resulted in a smaller increase in surface pressure for both PAMAM species binding to DPPG compared to the non-salt results, but only for PAMAM G4.5 this effect reached statistical significance (1.6 mN m^{-1} compared to 5.7 mN m^{-1} without the salt addition, $P < 0.05$). The decrease in penetration ability might be explained by sodium ions screening the anionic lipid heads and anionic surface groups of the PAMAM G4.5 and therefore reducing the electrostatic forces involved in the lipid layer penetration.

Discussion

We used two related biophysical techniques to study the binding behaviour of anionic PAMAM G4.5 and cationic PAMAM G5 to zwitterionic DPPC and anionic DPPG monolayers. To our knowledge, existing membrane studies of PAMAM dendrimers have been mainly focussing on cationic dendrimers [26, 27, 29] as due to their charge they have been proven good agents for drug [3, 4] and gene delivery [7, 8]. Interestingly, fewer studies have been carried out using anionic dendrimers despite their lower cytotoxicity [25, 44], antimicrobial activity [21, 45] and suitability as potential drug delivery agents [46, 47]. Just recently, their advantages over the cationic counterparts have started to attract more interest in the research community and membrane interactions *in vitro* were studied with the view of their potential as an anionic drug carrier [48, 49]. However, the molecular processes underlying their interactions with biomembranes and their lower cytotoxicity are still not understood and very few studies have been focussing on elucidating anionic PAMAM's interaction mechanism with model lipid membranes [50, 51] or proteins [52, 53] so far.

As indicated previously, surface pressure measurements are particularly sensitive to monolayer penetration by molecules [33, 54, 55]. This is used to our advantage since this technique is able to selectively probe interactions that lead to lipid penetration rather than those that result in significant adsorption of material below the lipid layer. It is not a quantitative measure of adsorbed amount, and measurements have shown that interactions where adsorption of molecules is below a lipid monolayer with little or no lipid penetration lead to negligible changes in surface pressure [56].

It has been observed previously that polycations, including PAMAM G5, are toxic towards eukaryotic cells at concentrations that depend on cell line and exposure time [11, 25]. Our monolayer studies show evidence of concentration-dependent penetration for both anionic PAMAM G4.5 and cationic PAMAM G5 to a DPPC monolayer selected as a simplified eukaryotic model membrane. The penetration at higher concentrations (> 0.18 mg mL^{-1}) seem to match (and could potentially explain) the toxicity reported for cationic PAMAMs and, in fact, evidence of membrane disruption and pore formation has been observed previously using different membranes models [26, 31, 57, 58, 59]. Those studies and simulation studies [60, 61, 62] concluded that such mechanisms are dependent on concentration and size of the cationic dendrimers. However, those studies do not explain our results observed for the anionic, better-tolerated PAMAM G4.5 which displayed similar penetration levels into our model as the cationic counterpart. It has been previously suggested that anionic PAMAM either induce membrane defects and leakage (G1.5) or prevent those by increased lipid packing and local swelling (G4.5) of amphiphilic POPC bilayers [63, 64]. Another study directly compared anionic PAMAM G2.5 and cationic PAMAM G3 binding to DPPC bilayers provides similar conclusions about their different membrane interaction behaviour [50].

Those observations from the PAMAM interactions are supported by other studies that investigated diverse mechanisms of interaction for the binding of anionic and cationic polymers to zwitterionic lipids; specifically the PC head group, and revealed that anionic nanoparticles reduce fluidity of lipid surfaces [65, 66]. Wang et al. [67] suggested that the charge of the approaching polymer affects the tilt angle of the zwitterionic PC head group as a result of the positions of the N+ and P- charges on the lipid. Considering the molecular weight of PAMAM G4.5 dendrimers, only a small number of molecular contacts are required to deeply penetrate the lipid monolayer to reach the hydrophobic region and for an observable change in surface pressure to occur [34, 54].

It is also interesting to consider the behaviour of PAMAM dendrimers to these simple lipid surfaces in the context of their selectivity between bacterial and eukaryotic membranes (Figure 2a and b). For example, other studies have shown that full-generation PAMAM G5 and G3 dendrimers are toxic against prokaryotes (i.e. *Pseudomonas Aeruginosa*; *Staphylococcus Aureus*) and that higher concentrations were required to observe toxicity against eukaryotic cells (human corneal epithelial cells; human bone mesenchymal stem cells)[20, 22, 23]. Studies involving anionic PAMAM species confirmed a similar toxic effect towards bacterial membranes, however at higher concentrations compared to their cationic counterparts [21, 45]. Interestingly, there are only few studies on eukaryotic membranes assessing anionic PAMAM dendrimers, but all reports indicate no or little toxicity even at concentrations up to 1 mg mL^{-1} (human corneal epithelial cells; human glioma cells) [49, 68]. The different interaction kinetics of PAMAM G5 and PAMAM G4.5 observed in our study indicate different modes of penetration into the DPPG and DPPC lipid monolayers, which might be related to the distinctive toxicity behaviour of both PAMAM species reported in some of the studies mentioned above, and might be due to the lipid model used..

Our observations of the comparator polymer PEG 20,000 Da showing no penetration in the investigated model membranes can be attributed to its physico-chemical characteristics (non-charged, with a flexible, linear geometry) and support PEG's previously reviewed biocompatibility and non-toxic behaviour towards eukaryotic and prokaryotic cells [69, 70]. In fact, PEGylation of PAMAM dendrimers has been used previously as a strategy to decrease cationic PAMAMs toxicity [20, 22, 25]. However, findings of some other groups suggest that low molecular weight PEG (6000, 8000 g mol^{-1}) could cause defects and leakage of zwitterionic lipid vesicles (i.e. DPPC; DMPC; POPC) [71, 72]. The use of a higher molecular weight PEG in our study (20,000 g mol^{-1}) could be potentially responsible for this difference observed, as the impact of the terminal OH would not be as significant as in lower molecular weight derivatives.

Another reported factor influencing polymer-membrane interaction is the salt content of the surrounding media [62, 73, 74] and this is also of biological relevance considering the different salts regulating physiological processes in the human body [75, 76]. Therefore, we decided to investigate the effect of addition of 144 mM of sodium chloride to the phosphate buffer subphase as this concentration corresponds roughly to the physiological sodium concentration found extracellularly (e.g. blood plasma). We found that the sodium chloride addition to the buffer solution did not affect the interaction of both PAMAM G4.5 and G5 with zwitterionic DPPC monolayers but seemed to decrease the interaction with anionic DPPG. Lee and Larson [62] showed in simulation studies with DPPC and PAMAM G5 addition of higher salt concentrations (500 mM) decreased interaction of the dendrimer with the lipid bilayer, probably due to the electrolyte weakening electrostatic forces. The significantly lower interaction of anionic PAMAM G4.5 with DPPG under sodium chloride addition could probably be explained by a study by Böhme et al. [42], where they found counterions reducing the effective charge of PAMAM dendrimers. These results are further supported by findings of Zhao et al. [77] where POPG bilayer showed strong attractive interactions between lipid molecules and dense lipid packing driven interlipid counterion bridges in the bilayers and strong intra- and intermolecular hydrogen bonding that increased even further under sodium ion addition and led complexation of PG molecules. Considering sodium as counterion for the carboxylate surface groups and the anionic headgroup of DPPG, the excessive amount available after sodium chloride addition to the buffer is likely to screen and bind to the anionic groups and therefore drastically reducing the charge density driven interaction. The small decrease observed for PAMAM G5 against DPPG in the presence of NaCl would be consistent with electrostatic interactions (this was however shown to not be statistically significant ($P > 0.05$, Table 2)). It should be noted that variations in ionic strength could affect other parameters in addition to charge interactions, most notably they could potentially affect the size of the dendrimers. However, although PAMAM dendrimers are polyelectrolites, their size has been reported to be independent from ionic strength [78], and as such, our discussion on this aspect has focussed on the effect on charge.

Conclusions

In this study, the interactions of the dendrimers PAMAM G5 and G4.5 with DPPC and DPPG monolayers has been investigated, with a view to providing an insight into how the polymer, lipid and solution properties can influence polymer binding to biological membranes. To our knowledge, we are the first to report comparative cationic/anionic PAMAM-membrane interaction studies utilising lipid monolayers. Both, PAMAM G4.5 and G5 were able to differentiate between the zwitterionic and anionic lipid layers, however their interaction profiles suggest different mechanisms of dendrimer binding. The strong electrostatic attraction between PAMAM G5 surface groups and the DPPG head group allowed for adsorption and penetration of the polymer into the lipid layer.

Interestingly, the anionic polymer PAMAM G4.5 showed evidence of strong penetration into DPPG monolayers. We hypothesise that this process is driven by DPPG head repulsion and interaction with lipid tails and intercalating into the hydrophobic areas of the lipid monolayer. Both PAMAM species interact in a concentration-dependent manner with the zwitterionic DPPC monolayers, however, much higher concentrations than for DPPG monolayers are required to induce significant increase in surface pressure, which is characteristic for membrane penetration. Addition of sodium chloride to the buffer solution decreased the interaction with anionic DPPG monolayers for both PAMAM G4.5 and G5, but did not affect their interaction with DPPC.

To confirm this hypothesis, further experiments would need to be carried out that enable the position of the polymer within the lipid layer to be quantitatively determined. Further proof of our hypothesis, could be achieved by studying the polymer-membrane interactions in more complex model systems, such as mixed lipid systems or cell culture. Taken together, our results provide an insight into the mechanism of interaction between charged dendritic polymers and a lipid membrane surface. We have identified that binding and lipid penetration can occur via different mechanisms where both electrostatic and hydrophobic interactions appear to play a role.

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Declaration of Interest

The authors report no conflicts of interest.

Table 1. Characteristics of the PAMAM dendrimers used in the study.

	PAMAM (Poly(amidoamine))	
Generation	G4.5	G5
Molecular Weight (g mol^{-1})[a]	26,251.86	28,824.81
Charge (in methanol)	Anionic	Cationic
Charge density: ratio of number of amino groups to MW[b]	0.0048	0.0088 (0.0044)[c]

[a] As determined by the manufacturer

[b] Theoretical values

[c] Considering only surface amino groups

Table 2. Effect of presence of sodium chloride in the phosphate buffer subphase (pH 7, 20 mM) on interaction of PAMAM G5 and G4.5 with DPPG and DPPC monolayers. Δ surface pressure data indicate mean ± SEM. PAMAM dendrimer concentration for interacting with DPPG was 0.061 mg mL^{-1}; for interacting with DPPC 0.3 mg mL^{-1}.[#]

	Δ Surface Pressure / mN m^{-1}			
	PAMAM G5		**PAMAM G4.5**	
	DPPG	**DPPC**	**DPPG**	**DPPC**
0 mM NaCl	4.3 ± 0.3	3.1 ± 0.2	5.7 ± 0.6	4.0 ± 0.4
144 mM NaCl	3.1 ± 0.4 ns	3.8 ± 0.8 ns	1.6 ± 0.2 *	3.6 ± 0.3 *

[#] statistical significance (Student's t test 144 vs 0 mM) indicated; ns = non-significant, * = P ≤ 0.05

Figure 1. Chemical structures of the lipids and the polymers used in this study. a) Chemical structure of i) 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); ii) 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DPPG). b) Chemical Structure of linear polyethylene glycol (PEG). c) Chemical structure of poly(amidoamine) (PAMAM) dendrimer with ethylenediamine core; generations inset: i) PAMAM G4.5: R-group anionic in solution (pH 7), sodium carboxylate surface groups; ii) PAMAM G5: R-group cationic in solution (pH 7), amino surface groups.

Figure 2. Selectivity of polymers towards different lipid monolayers. Changes in surface pressure for PEG 20,000 (panel a); PAMAM G5 (panel b); and PAMAM G4.5 (panel c) binding to DPPG (filled symbols) and DPPC (open symbols) monolayers were recorded over time. Experiments were carried out at pH 7, surface pressure was at 21 ± 2 mN m^{-1} immediately prior to polymer addition at concentration 0.061 mg mL^{-1}. Data show profiles for a single representative experiment from a set of at least 3 independent experiments (n≥3). Panel d shows comparison of maximum increase in surface pressure observed for the 3 polymers against DPPG (black) and DPPC (white), data show mean ± SEM (n≥3). Statistical significance calculated via Student's t test, and set at P≤ 0.05. ns = non-significant.

Figure 3. Concentration-dependence of polymer interactions with lipid monolayers. Binding of PAMAM G5 (panel a); PAMAM G4.5 (panel b); and PEG 20,000 (panel c) to DPPG (filled symbols) and DPPC (open symbols) was studied at pH 7. Surface pressure was 21 ± 2 mN m^{-1} prior to polymer addition. Maximum surface pressure change data displayed indicate mean ± SEM; error bars are hidden by the symbol when not visible. Statistical significance calculated via Student's t test, and set at P≤ 0.05. ns = non-significant. Comparisons carried out are highlighted with dashed line.

Figure 4. Concentration-dependent effect of PAMAM G5 dendrimers on the Amide I region of the FTIR spectrum. Changes in the peak area of the Amide I (panel a) were observed upon dendrimer binding to DPPG (filled symbols) and DPPC (open symbols) monolayers. The inset enlarges the data for interaction with DPPG only. Exemplary spectra focusing on the Amide I region (panel b) showing the addition of PAMAM G5 to a DPPG monolayer in the condensed phase. i) 0.0024 mg mL^{-1}; ii) 0.0125 mg mL^{-1}; 0.018 mg mL^{-1}; iv) 0.024 mg mL^{-1}. No visible changes were associated to the C = O

stretch of the headgroup of the phospholipid. Reproducibility of the technique has been verified via experiments with a subset of PAMAM dendrimers of different generations.

Figure 1

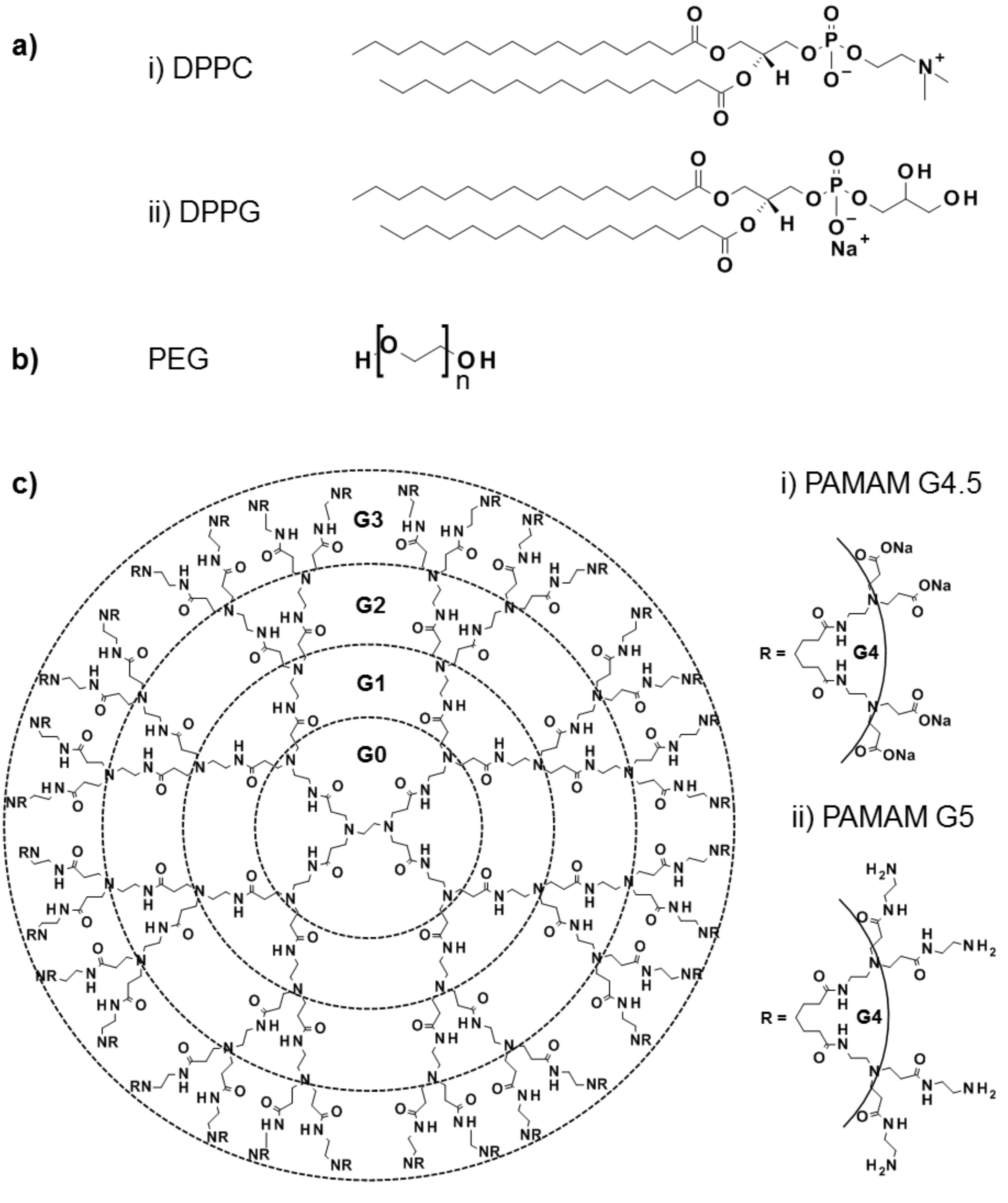


Figure 2

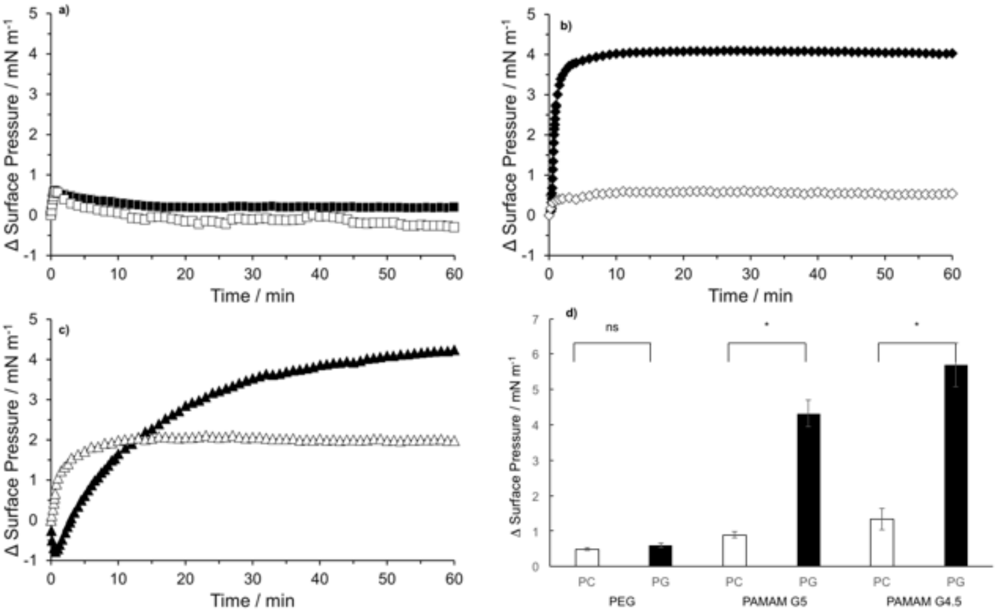

a)
b)
c)
d)
Δ Surface Pressure / mN m^{-1}
Time / min
5
4
3
2
1
0
-1
0
10
20
30
40
50
60
7
6
ns
*
*
PC
PG
PEG
PAMAM G5
PAMAM G4.5

Figure 3

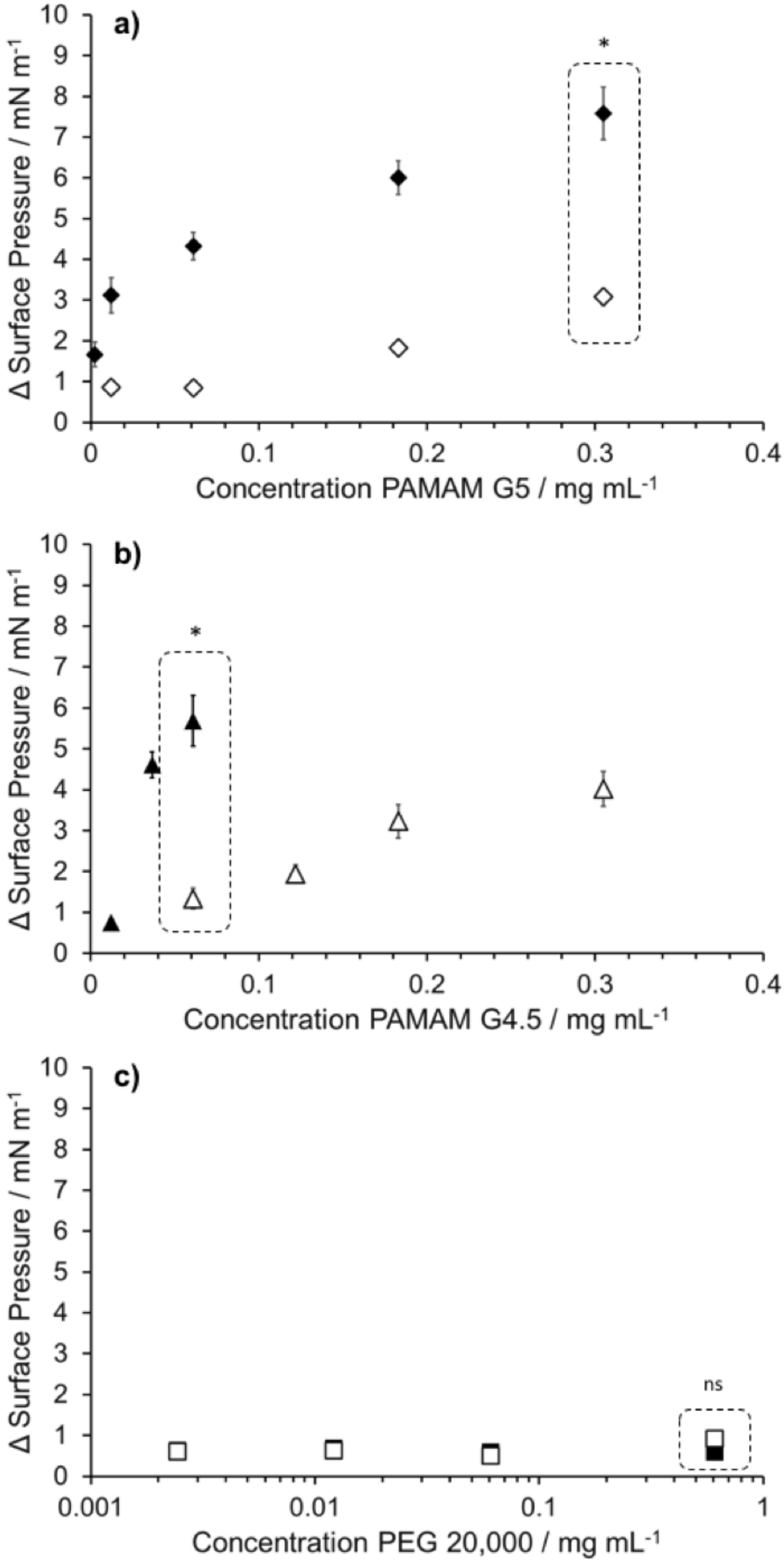
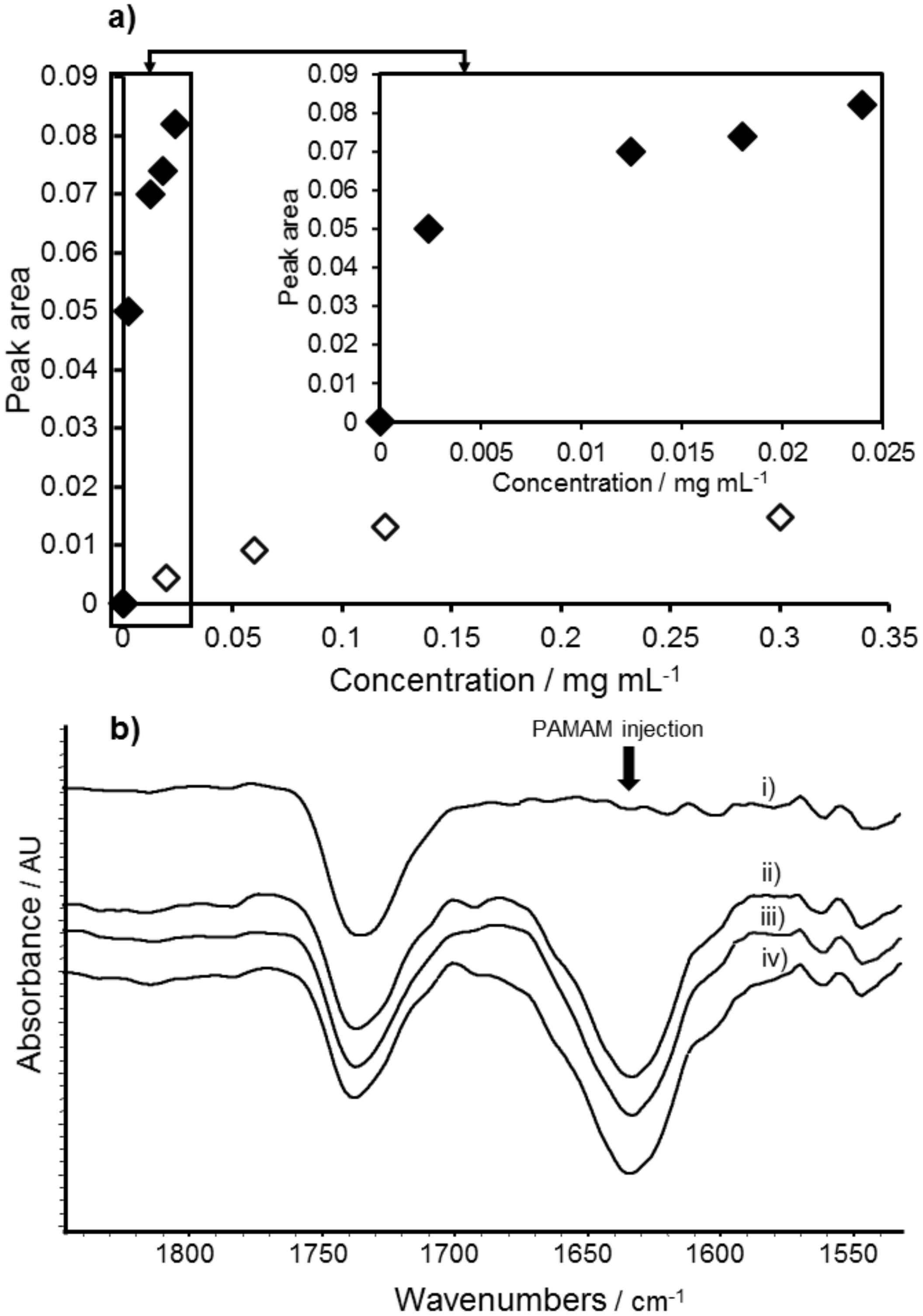

a)
*
Δ Surface Pressure / mN m^{-1}
0
1
2
3
4
5
6
7
8
9
10
0
0.1
0.2
0.3
0.4
Concentration PAMAM G5 / mg mL^{-1}
b)
*
Δ Surface Pressure / mN m^{-1}
0
1
2
3
4
5
6
7
8
9
10
0
0.1
0.2
0.3
0.4
Concentration PAMAM G4.5 / mg mL^{-1}
c)
ns
Δ Surface Pressure / mN m^{-1}
0
1
2
3
4
5
6
7
8
9
10
0.001
0.01
0.1
1
Concentration PEG 20,000 / mg mL^{-1}

Figure 4


a)
Peak area
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
Concentration / mg mL^{-1}
Peak area
0.005
0.01
0.015
0.02
0.025
Concentration / mg mL^{-1}
b)
PAMAM injection
i)
ii)
iii)
iv)
Absorbance / AU
1800
1750
1700
1650
1600
1550
Wavenumbers / cm^{-1}

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