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PAMAM dendrimers and model membranes: Differential scanning calorimetry studies

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Abstract

Dendrimers attract much attention as potential drug and gene carriers for intracellular delivery. From this point of view, it is crucial to extend our knowledge about their interactions with membranes.

The influence of polyamidoamine (PAMAM) dendrimers on the thermotropic behavior of DPPC multilamellar vesicles and DMPC small unilamellar vesicles was examined by differential scanning calorimetry. We used three types of PAMAM dendrimers to determine how a dendrimer structure determines interactions with liposomes.

We show that the strength of interactions depends on both the dendrimers' structure and degree of hydrophobicity. A model for the interaction of each type of dendrimer with liposomes was proposed.

Keywords: Dendrimer; PAMAM; Liposome; Vesicle; Membrane; DSC; MLV; SUV

1. Introduction

Dendrimers are a relatively new class of polymers. They were first synthesized in the mid-1980s by Tomalia's group (Tomalia et al., 1985). Since then they have attracted much interest due to their specific structure and unique properties. All dendrimers are built from a central core molecule, which is surrounded by layers of branched monomers. As a result, the dendrimers adopt globular shape with a densely packed surface and empty internal cavities (Tomalia et al., 1990). The more layers that are attached, the higher the so-called dendrimer's generation is. Among the possible applications of dendrimers, the medical ones seem particularly important. One potential medical application is their use as efficient transfection agents (Haensler and Szoka, 1993; Bielinska et al., 1996; Wang et al., 2000). They transport more genetic material than liposomes and do not cause a strong immunological response as viral vectors do. Second, dendrimers can play an important role as drug-delivery systems. Drug molecules can be attached to end groups or encapsulated in a dendrimer's interior (Zhuo et al., 1999; Kojima et al., 2000).

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The possibility of using dendrimers in medicine has raised a motivation for basic scientific investigations. Interactions between dendrimers and membranes are important for the development of dendrimer-mediated drug-delivery systems, so that dendrimers can assist in the passage of a drug through a cell membrane. As well in the case of transfection, it is the membrane that presents a barrier for the entry of nucleic acid into the cell, and therefore an understanding of the interactions between dendrimers and bilayers is important.

Liposomes are widely employed as models of biological membranes and there have been some studies of their interactions with dendrimers. In particular, the interaction of polyamidoamine dendrimers with dimyristoylphosphatidylcholine (DMPC) has been most extensively studied. It was found that the strength of interaction depended on a size of the dendrimer and whether it is charged or not. Protonated dendrimers showed more effective dendrimer–vesicle interactions and higher generation dendrimers created more disturbances (Ottaviani et al., 1998,1999). Generally dendrimers did not significantly perturb DMPC membrane properties, but they were able to disrupt anionic vesicles (Zhang and Smith, 2000). Studies of mixed vesicles composed of DMPC and the negatively charged dimyristoylphosphatidic acid (DMPA)

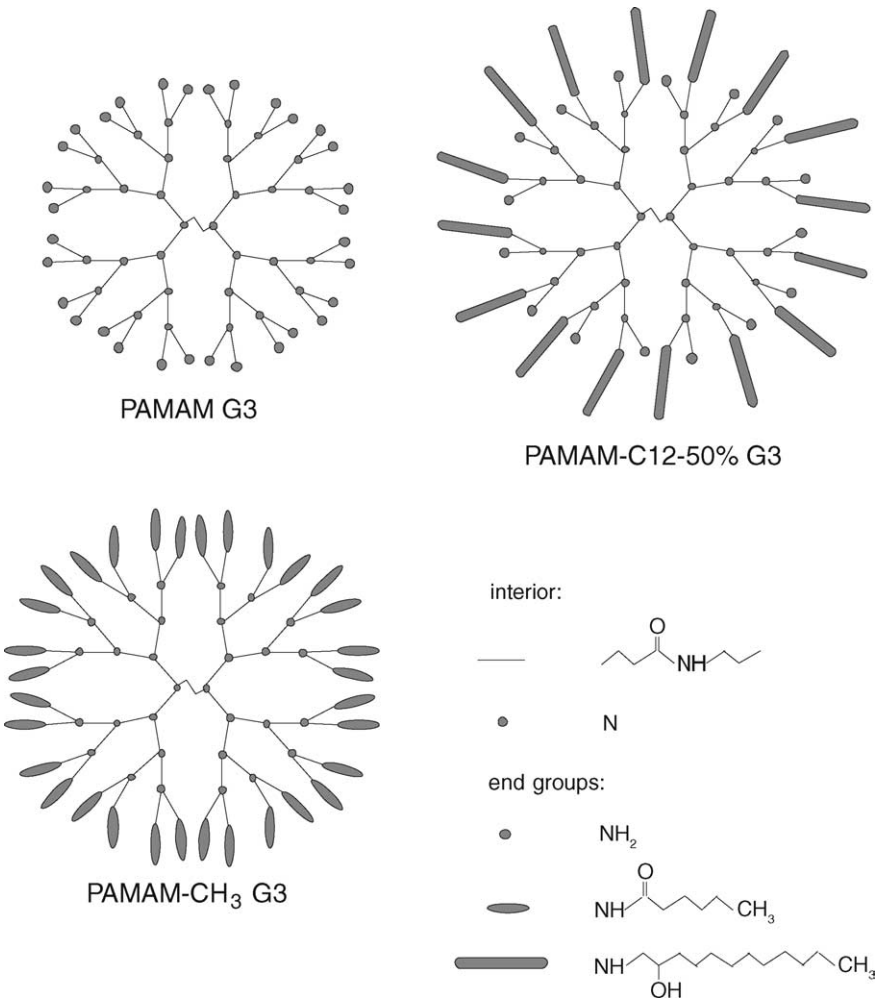

Fig. 1. Schematic structures of studied dendrimers.

was suggested to result in the lipid bilayer wrapping around the dendrimer (Ottaviani et al., 2002). It was found that perturbation of membranes by dendrimers is strongly dependent on membrane lipid composition. Phosphatidyletanolamine (PE) vesicles were disrupted, but phosphatidylcholine (PC) vesicles and even mixed vesicles with a high fraction of PC were protected from disruption (Zhang and Smith, 2000). The outer surface of large dendrimers is too densely packed to be penetrated by phospholipids. It was proposed that PAMAM preferentially interact with lipids having a greater negative curvature tendency. Membranes containing a high PE content can wrap around the dendrimer surface. Dendrimers mediate both the contact between vesicles, and may also promote the creation of a local region of hexagonal phase (Karoonuthaisiri et al., 2003). For DMPC lipid bilayers an alternative hypothesis was suggested according to which dendrimers can enter into a cell through membrane pores. The hypothesis was based on atomic force microscopy studies that showed that dendrimers create small holes in a bilayer and on leakage experiments (Hong et al., 2004).

Previously the main experimental methods employed to study interactions between dendrimers and lipid bilayers were ^{31}P NMR spectroscopy to monitor changes in membrane morphology, EPR and spectrofluorimetric methods to measure membrane fluidity, and leakage and mixing assays to study fusion processes. In our studies we determined the influence of three different types of dendrimers on the thermotropic behavior of DPPC multilamellar vesicles (MLVs) and DMPC small unilamellar vesicles (SUVs) using differential scanning calorimetry (DSC). Monitoring phospholipid phase transitions with DSC is a sensitive method for studying changes in model membranes induced by different substances that interact with lipid. We chose these lipids because they have been extensively studied in model membranes. They also exhibit phase transitions at temperatures that are convenient to monitor with DSC.

We used three types of polyamidoamine dendrimers to determine how a dendrimer structure determines interactions with liposomes. Polyamidoamine (PAMAM) dendrimers are based on an ethylenediamine core, and branched units are constructed from both methyl acrylate and ethylenediamine. The dendrimers used in this work are PAMAM-C12-50% dendrimer which possesses 50% of amino groups and 50% of *N*-(2-hydroxydodecyl) groups, PAMAM-CH_3 a dendrimer having hexylamide surface groups, and an amino-terminated PAMAM dendrimer. All these dendrimers are the third generation ones (G3) and they have 32 end groups on their surfaces. Molecular weights for PAMAM-C12-50%, PAMAM-CH_3 and PAMAM are 12807 Da, 10050 Da and 6909 Da, respectively. Their schematic structures are shown on Fig. 1. PAMAM dendrimer is the only one which is water-soluble. Two other dendrimers are hydrophobic and they are characterized by different shapes: flat, ellipsoidal molecules of PAMAM-CH_3 and larger PAMAM-C12-50% dendrimers with long hydrocarbon chains on the surface.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further purification. PAMAM dendrimer, PAMAM-CH_3 dendrimer and PAMAM-C12-50% dendrimer (generation 3) were purchased as solutions in methanol from Sigma–Aldrich (Oakville, Ont.). PIPES buffer (pH 8.0) used to hydrate films contained: 20 mM pipes, 0.14 M NaCl, 1 mM EDTA and 20 mg/L NaN_3. All of these chemicals were of analytical grade. Water used to prepare solutions was double-distilled and deionised.

2.2. Preparation of dendrimer-containing MLVs

2.2.1. Method A

The appropriate amounts of DPPC and dendrimers were co-dissolved in chloroform–methanol (2:1, v/v). The solvent was evaporated under a stream of nitrogen and then samples were placed under vacuum for 2 h to remove any traces of residual solvent. PIPES buffer was added to the dry film and hydrated to give a final DPPC concentration of 2.72 mM. Multilamellar vesicles (MLVs) were obtained by shaking a tube on a vortex mixer for approximately 2 min (sufficient time for the lipid on the wall to become suspended in buffer) and then the sample was subjected to five freeze–thaw cycles in which dispersions were frozen in liquid nitrogen and then thawed at 50 °C in a water bath. Between the each cycle the sample was vortexed for 15 s at a

temperature above the main transition temperature of pure DPPC.

2.2.2. Method B

The stock solution of dendrimers in methanol was added to a tube. The methanol was removed with a stream of nitrogen and a thin dendrimer film was formed on the wall of the tube. Further evaporation was carried out by keeping the sample under vacuum for 2 h. Next, the dendrimer film was hydrated with PIPES buffer.

DPPC was dissolved in chloroform–methanol (2:1, v/v) and a DPPC film was prepared as described above and dispersed by adding the solution of dendrimers to obtain the appropriate concentration. Multilamellar vesicles were prepared in the same way as in method A.

2.2.3. Method C

The DPPC dry film, instead of hydrating with dendrimer solution, was suspended with PIPES buffer. The procedure to obtain MLVs was analogous to method A. After obtaining multilamellar liposomes, a solution of dendrimer in buffer was added and sample was vortexed to ensure mixing.

Methods B and C could be employed only with the PAMAM dendrimer because this type of dendrimer is the only water-soluble one.

2.3. Preparation of dendrimer-containing SUVs

The appropriate amounts of DMPC and dendrimers were co-dissolved in chloroform–methanol (2:1, v/v). The solvent was evaporated under nitrogen stream and then samples were placed under vacuum for 2 h to remove any traces of residual solvent. PIPES buffer was added to the dry film to hydrate to a final DMPC concentration of 2.95 mM. Multilamellar vesicles (MLVs) were obtained by shaking a tube on a vortex mixer for 2 min (to suspend the lipid). To produce SUVs, the MLV dispersion was sonicated for 2 h in a bath type sonicator under argon at room temperature. To study the amino-terminated PAMAM dendrimer, three methods of adding dendrimers were employed as were used in case of MLVs.

2.4. DSC measurements

All samples were degassed before measurement. When samples had been incubated at 65 °C, they were allowed to cool down to room temperature before being loaded into the calorimeter.

The DSC measurements were performed using MicroCal VP-DSC calorimeter. 0.5 mL liposome solution was placed in the sample cell. All thermograms were run using the same volume of PIPES buffer as a reference. Samples were heated and cooled repeatedly five times at a rate 30°/h between 15 °C and 65 °C in the case of DPPC MLVs, and between 5 °C and 40 °C in the case of DMPC SUVs. There was a delay of 5 min between sequential scans to allow for thermal equilibration.

3. Results

3.1. Interactions between DPPC MLVs and amino-terminated PAMAM dendrimers

Dendrimers were added to DPPC at three different concentrations: 0.1 mol%, 1 mol%, and 10 mol%. When liposomes with dendrimers were prepared as described in method A, amino-terminated dendrimers caused only small changes in the thermotropic behavior of DPPC (Table 1). For the lowest dendrimer concentration both the main phase transition and the pretransition were not significantly altered. Increasing the PAMAM dendrimer concentration resulted in a slight broadening of the main transition peak (widths at a half-height changed from 0.2° for pure DPPC to around 0.4° in the presence of dendrimers). This alteration was accompanied by a decrease in the pretransition enthalpy that was much smaller than for pure DPPC. The highest concentration of dendrimer caused a decrease in the pretransition temperature from 34.5 °C to 32.5 °C. It is interesting that the pretransition enthalpy decreased after each heating and cooling cycle (Fig. 2). To check if keeping the sample at a temperature above the gel to liquid-crystalline phase transition resulted in the progressive decrease of the pretransition, the samples were incubated at 65 °C for 24 h and then loaded into the calorimeter. In the case of pure DPPC, the incubation did not cause a significant effect. However, for systems with dendrimers the changes were much visible even for the lowest dendrimer concentration (Table 2). The half-height widths of both transition endotherms were broadened, the enthalpy of the main transition was lower

Table 1
Calorimetric data obtained for PAMAM G3–DPPC MLVs solutions prepared according to different methods

Dendrimer concentration (mol%)	Scan number	Heating						Cooling		
		Main transition			Pretransition			Main transition		
		ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)
		7.5[a]	41.2[a]	0.21[a]	1.0[a]	34.5[a]	1.89[a]	−7.2[a]	40.8[a]	0.25[a]
Method A										
0.1	1st	7.5	41.2	0.21	1.0	34.7	2.01	−7.2	40.8	0.20
	5th	7.6	41.2	0.25	0.9	34.1	2.14	−7.3	40.6	0.24
1	1st	7.8	41.2	0.25	0.9	34.6	1.72	−7.6	40.6	0.45
	5th	7.9	40.9	0.46	0.1	33.7	3.64	−7.7	40.5	0.54
10	1st	7.5	41.2	0.29	0.9	34.3	2.18	−7.2	40.7	0.29
	5th	7.5	41.0	0.38	0.4	32.4	2.93	−7.3	40.6	0.33
Method B										
0.1	1st	7.2	41.2	0.21	0.9	35.1	1.62	−6.1	40.8	0.29
	5th	6.0	41.1	0.87	0	–	–	−6.2	40.3	0.84
1	1st	7.2	41.2	0.21	0.9	35.1	1.56	−6.3	40.7	0.29
	5th	6.4	40.9	0.75	0	–	–	−6.4	40.6	0.50
10	1st	7.6	41.1	0.21	0.8	34.9	1.68	−6.6	40.7	0.29
	5th	7.0	41.1	0.87	0	–	–	−6.5	40.2	0.96
Method C										
0.1	1st	7.5	41.2	0.21	1.0	34.6	2.10	−6.8	40.8	0.29
	5th	7.3	41.0	0.59	0.1	34.8	4.81	−7.2	40.6	0.71
1	1st	7.7	41.2	0.21	1.0	34.5	2.02	−6.3	40.8	0.33
	5th	7.4	40.8	0.79	0	–	–	−6.7	40.4	0.67
10	1st	7.4	41.2	0.25	0.7	32.5	2.18	−7.0	40.7	0.54
	5th	6.6	40.6	1.21	0	–	–	−7.2	40.2	0.88

[a] Control-pure DPPC

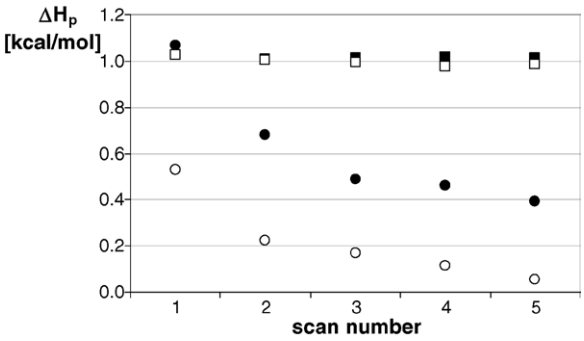

Fig. 2. Changes of the pretransition enthalpy with a heating scan number for pure DPPC (■) and DPPC incubated at 65 °C for 24 h (□); and upon addition of 10 mol% of PAMAM G3 dendrimer, without incubation (●) and after incubation at 65 °C for 24 h (○).

and a marked decrease of the pretransition peak was observed (Fig. 3A). Nevertheless progressive changes were still observed with an increasing number of scans (Fig. 2).

As PAMAM dendrimers are water-soluble polymers, it was possible to check their impact on the thermal behavior of DPPC when they were added as aqueous solutions to lipid films at the hydratation stage (method B) or added after the preparation of MLVs (method C). For both methods B and C changes in thermograms were larger than for method A (Fig. 4). When dendrimers were added after MLVs had been prepared, the main phase transition peak became asymmetric with skewing toward lower temperatures. Keeping a dendrimer–liposome solution prepared according

Table 2
Calorimetric data obtained for samples that were incubated at 65 °C for 24 h

Dendrimer concentration (mol%)	Scan number	Heating						Cooling		
		Main transition			Pretransition			Main transition		
		ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)
		8.2[a]	41.2[a]	0.34[a]	1.0[a]	33.7[a]	2.55[a]	−7.7[a]	40.8[a]	0.33[a]
Method A										
0.1	1st	7.4	41.1	0.50	0.8	32.8	3.51	−6.3	40.7	0.50
	5th	7.2	40.9	0.83	0.1	30.6	3.77	−6.4	40.5	0.42
1	1st	6.5	41.1	0.42	0.6	32.9	3.18	−5.8	40.6	0.46
	5th	5.9	40.8	0.91	0	–	–	−6.4	40.4	1.09
10	1st	7.5	40.4	0.67	0.5	29.8	2.69	−7.3	40.1	0.55
	5th	6.9	40.3	0.80	0	–	–	−7.5	39.9	0.42
Method C										
0.1	1st	7.7	41.2	0.29	0.9	34.0	2.25	−6.7	40.8	0.29
	5th	6.6	41.0	0.63	0.1	32.3	3.46	−6.7	40.6	0.75
1	1st	7.4	41.1	0.38	0.8	32.2	2.88	−6.9	40.7	0.37
	5th	7.1	40.9	0.83	0	–	–	−7.1	40.5	0.84
10	1st	7.2	40.1	0.83	0.3	28.6	2.43	−7.0	39.6	0.71
	5th	7.3	39.6	2.05	0	–	–	−7.0	39.3	0.83

PAMAM G3–DPPC MLVs solutions were prepared according to different methods.
[a] Control-pure DPPC

to method C in bath at 65 °C for 24 h did not increase the changes (Fig. 3B).

3.2. Interactions between DPPC MLVs and methyl-terminated PAMAM-CH_3 dendrimers

Dendrimers at three different concentrations: 0.1 mol%, 1 mol%, and 10 mol%, were dissolved with DPPC in chloroform:methanol (2:1) and made into a lipid film. These concentrations were the same as those for the amino-terminated PAMAM dendrimers.

The character of the changes in lipid phase transition behavior was similar as for water-soluble dendrimers, but the extent of change with PAMAM-CH_3 dendrimers with a lipid bilayer was greater than for PAMAM (Table 3). Again the temperature of the main transition remained nearly the same but peaks were broadened and the pretransition was almost lost (Fig. 5). Moreover, changes were progressive with scan number. Surprisingly, for the highest tested dendrimer concentration (10 mol%) the disturbances were much smaller than for concentrations 0.1 mol% and 1 mol%.

3.3. Interactions between DPPC MLVs and PAMAM-C12-50% dendrimers

All thermotropic parameters were significantly altered by the presence of PAMAM-C12-50% dendrimers. Contrary to the previously described more polar dendrimers, sequential scans showed good reproducibility. Dendrimers affected both the main phase transition and the pretransition. A concentration of PAMAM-C12-50% dendrimers necessary to diminish the pretransition was much lower than for two other tested dendrimers and equaled 2 mol% (Table 3). The main transition was broader, smaller and slightly shifted toward lower temperatures upon addition of dendrimers. The higher the concentration of dendrimers was, the bigger disturbances were observed (Fig. 6). The enthalpy of the main transition decreased with increasing concentration and for a concentration of 2 mol% dendrimer the enthalpy equaled 2.8 kcal/mol. A width at a half-height changed from 0.2° for pure DPPC to around 0.8° in the presence of dendrimers.

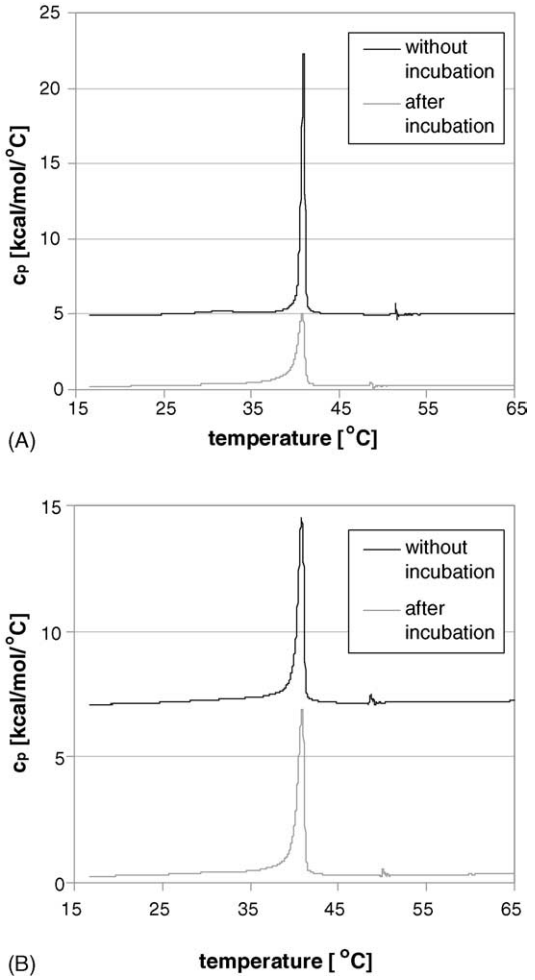

Fig. 3. Heating scans for DPPC MLVs in the presence of 1 mol% of PAMAM G3 dendrimer added according to the method A (graph A) and method C (graph B) without incubation and after 24 h of incubation at 65 °C. All scans are after four cycles of heating and cooling and they correspond to the equilibrium state.

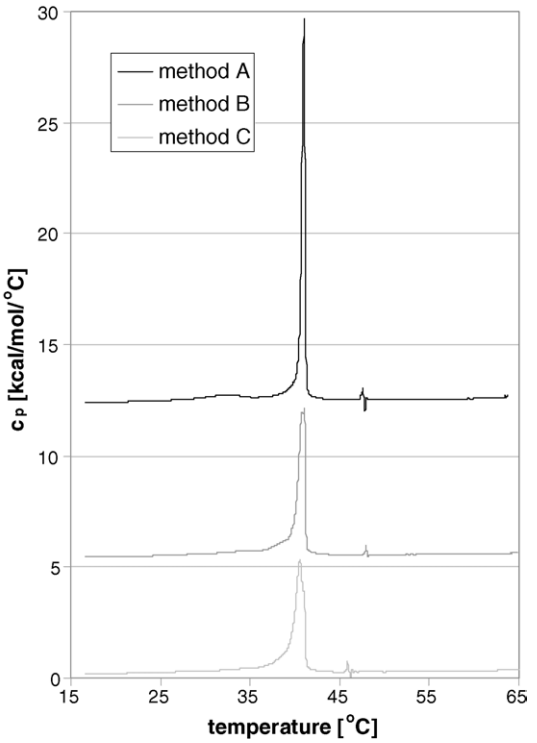

Fig. 4. Comparison of different methods of preparation of liposome–dendrimer solutions. DPPC MLVs heating scans upon addition of 10 mol% of PAMAM G3 dendrimer. All scans are after four cycles of heating and cooling and they correspond to the equilibrium state.

3.4. Interactions between DMPC SUVs and dendrimers

To study interactions between small unilamellar vesicles (SUVs) and dendrimer, we used DMPC instead of DPPC. Dipalmitoyl phosphatidylcholine (DPPC) possesses 16 carbons in each acyl chain and dimyristoyl phosphatidylcholine (DMPC) has two carbons less. The shorter acyl chains, the lower the temperature of the main transition.

The DSC thermograms for SUVs are considerably different when they are compared to ones obtained for MLVs. It is known that the cooperativity of the phase transition decreases as the vesicle curvature increases (Suurkuusk et al., 1976).

The impact of hydrophilic dendrimers on SUVs depended on the method of preparation. No change was observed when dendrimers were added after SUVs were formed (Table 4). The biggest disturbances were noticed when the lipid film was hydrated with a dendrimer solution (Fig. 7). It is interesting that similar scans were obtained upon addition of PAMAM-C12-50% dendrimers and PAMAM dendrimers when solutions were prepared according to method A. The most asymmetric shape of a thermogram was observed for PAMAM-CH_3 dendrimers.

Table 3
Calorimetric data obtained for PAMAM-CH_3 G3–DPPC MLVs solutions and PAMAM-C12-50% G3–DPPC MLVs solutions

Dendrimer concentration (mol%)	Scan number	Heating						Cooling		
		Main transition			Pretransition			Main transition		
		ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)
PAMAM-CH_3 G3										
0.1	1st	7.6	41.1	0.42	1.0	33.5	2.64	−6.9	40.7	0.33
	5th	7.7	40.1	0.79	0.1	30.1	3.99	−7.6	40.3	0.71
1	1st	7.3	41.1	0.42	0.9	33.3	2.80	−7.0	40.6	0.41
	5th	7.2	40.9	0.83	0.1	32.1	3.73	−6.8	40.5	0.75
10	1st	7.8	41.1	0.25	0.8	32.9	2.01	−7.5	40.7	0.25
	5th	7.5	41.1	0.38	0.4	33.4	2.30	−7.3	40.6	0.37
PAMAM-C12-50% G3										
0.1		6.3	41.1	0.33	0.5	33.7	2.51	−6.2	40.7	0.29
0.5		4.7	40.9	0.54	0.1	33.5	2.13	−4.8	40.4	0.59
2		2.6	40.7	0.75	0	–	–	−2.6	40.2	0.75

Table 4
Calorimetric data obtained for DMPC SUVs upon addition of dendrimers (concentration 1 mol%)

Dendrimer	Heating			Cooling		
	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)	ΔH (kcal/mol)	T_m (°C)	$T_{1/2}$ (°)
Control–pure DMPC	3.0	22.8	1.60	−2.2	22.3	1.83
PAMAM G3 method A	3.7	22.5	1.72	−4.2	22.0	2.27
PAMAM G3 method B	1.5	20.3	2.06	−2.6	19.4	3.69
PAMAM G3 method C	3.6	22.7	1.77	−2.8	22.2	2.10
PAMAM-CH_3 G3	5.2	23.1	3.48	−4.8	22.7	3.06
PAMAM-C12-50% G3	3.5	22.6	1.64	−4.5	22.2	2.17

4. Discussion

The purpose of our studies was to investigate the interactions of dendrimers with phospholipids in a model bilayer system. We studied the influence of three different types of dendrimers on the thermotropic behavior of DPPC MLVs and DMPC SUVs using differential scanning calorimetry to determine the relationships between the structure and hydrophobicity of dendrimers on their effect on membranes.

During heating DPPC liposomes exhibit two endothermic transitions: a broad pretransition with a low enthalpy and sharp main transition. The pretransition corresponds to the conversion of a lamellar gel phase to a rippled gel phase. The main transition is a consequence of the conversion of a rippled gel phase to a liquid-crystal phase.

We determined thermotropic properties of DPPC multibilayers such as: enthalpies for the main phase transition and the pretransition, maximal temperatures of transition endotherms and widths of peaks at a half-height that correspond to the cooperativity of the transition. In pure DPPC liposomes, the values we obtained for the main transition and for the pretransition are in a good agreement with those reported in the literature (Huang and Li, 1999). The exothermic calorimetric plots measured during cooling cycles usually confirmed the results obtained during heating scans but were of opposite sign, as expected.

Generally, the pretransition was the most sensitive to the presence of all types of dendrimers. The pretransition enthalpy was significantly lower compared to the dendrimer-free samples. A similar effect was observed

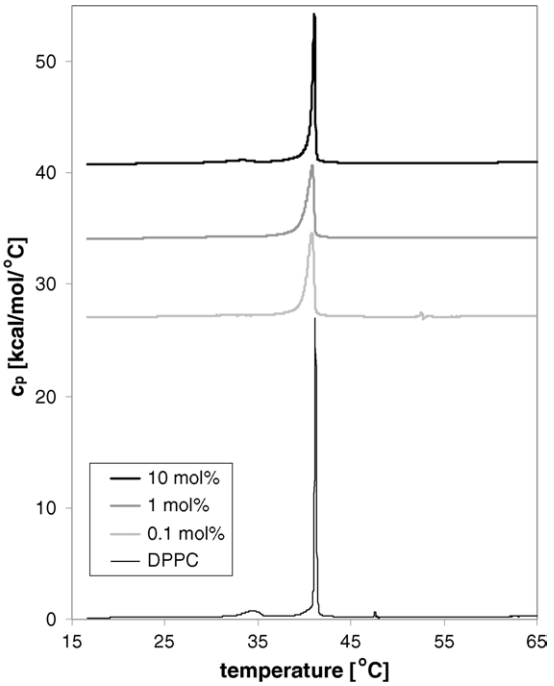

Fig. 5. The impact of PAMAM-CH_3 dendrimer on DPPC MLVs heating scans. All scans are after four cycles of heating and cooling and they correspond to the equilibrium state.

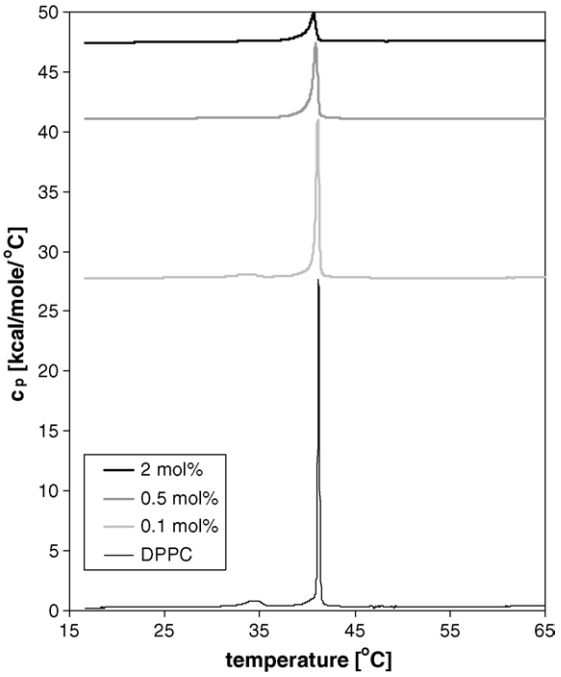

Fig. 6. The impact of PAMAM-C12-50% dendrimer on DPPC MLVs heating scans.

for interactions between poloxamer surfactants and DPPC MLVs (Castile et al., 1999). It is well known that the pretransition is very sensitive to the presence of "foreign" substances and can disappear when a very small amount of them is added (Bonora et al., 2002). The disappearance of a pretransition means that upon addition of dendrimers the conversion between rippled and lamellar gel phase was abolished. This is likely a consequence of the dendrimer inserting into the bilayer to increase the spacing between phospholipid molecules and eliminating steric crowding of the PC headgroup, thus eliminating the driving force for the formation of a ripple phase.

Usually, if there are "foreign" molecules in a bilayer, they affect the cooperativity of the phase transition. The analysis of the peak width at half-height reveals the difference in the extent of cooperativity. In our case, the largest decrease in the cooperativity was observed in the presence of PAMAM-C12-50% dendrimers. This is probably due to the presence of the long hydroxydodecyl chains on the surface of dendrimers that could

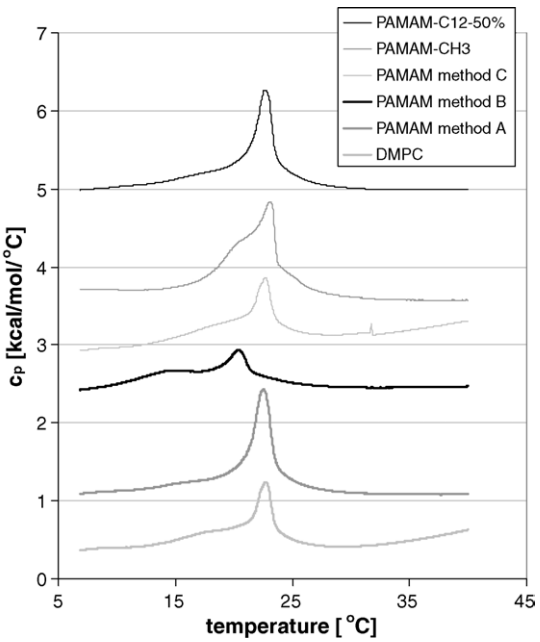

Fig. 7. DMPC SUVs heating scans upon addition of 1 mol% of dendrimer.

insert deeply into hydrophobic core of bilayer reducing the transition cooperativity and causing the disappearance of the pretransition.

Many parameters changed gradually during successive DSC scans. To determine if this was a result of greater penetration of the dendrimer into the bilayer at higher temperature, we incubated samples at 65 °C for 24 h before running the DSC. We observed that the incubation caused a broadening of the main transition peak and it was slightly shifted to lower temperatures. We also observed the disappearance of the pretransition. Nevertheless, incubation in high temperature had no significant effect when dendrimers were added after MLVs were formed. It is interesting that even after long incubation at 65 °C many parameters still changed after each heating/cooling cycle. Usually the changes were less pronounced with each scan and for fourth and fifth scans they were almost negligible. It thus appears likely that phase boundary defects that occur in the temperature region of the phase transition facilitate the attainment of equilibrium, rather than incubation at higher temperatures. Defects form during the several crossings of the phase transition temperature in the DSC, allowing greater penetration of the dendrimer into the bilayer. A similar phenomenon was observed earlier for interactions between DMPC liposomes and poloxamers (Castile et al., 1999). Hydroxy-terminated PAMAM-OH dendrimers also facilitated transport of SYTOX Green dye across the bacterial cell membrane (Chang et al., 2001). Moreover, recently it has been shown that PAMAM dendrimers are able to create holes within DMPC membranes (Mecke et al., 2004), suggesting that the permeability of lipid bilayer can be changed as a result of the presence of dendrimers that bind to the bilayer. This can have important implications for drug delivery.

It has been found that the method of preparation of dendrimer–liposome solutions had a large impact on the final product. The thermal behavior of MLVs was the least changed when dendrimers were added to lipid solutions in methanol:chloroform, and the biggest when dendrimers were added after liposome preparation. In methods A and B, dendrimers were frozen and thawed together with lipids. It is known that freeze-thawing improves a penetration of exogenous molecules throughout MLVs. This happens because of transient damage to the bilayer as a consequence of ice crystal formation as well as increased penetration of solute into the core of the liposome (Gruner et al., 1985; Mayer et al., 1985). In our experiments, repeated freeze-thawing cycles resulted in the dendrimers being more uniformly distributed. When dendrimer–liposome suspensions were prepared according to method A, the phase transitions were symmetrical, which supports the idea of dendrimers being uniformly located within bilayer. When dendrimers were added after the liposome preparation we observed the occurrence of an asymmetric main phase transition peak. This can be explained as a result of a non-uniform distribution of dendrimer molecules and the formation of dendrimer-rich and dendrimer-poor domains. Such a phenomenon was observed earlier, e.g. for carotenoids (Kostecka-Gugala et al., 2003). Moreover, it is known that “foreign” molecules that are located on the bilayer surface have a stronger influence on the pretransition and the main transition peaks than those that are uniformly located in hydrophobic interior (Shimshick and McConell, 1973). This is in a good agreement with our results. It indicates that freeze-thawing cycles reduced the percentage of aggregated dendrimers. When dendrimers were added after liposome preparation, the dendrimers’ adsorption on the bilayer surface was probably the predominant phenomenon.

We found that the extent of thermotropic perturbations strongly depended on the type of dendrimers and on their concentrations. Both in the case of amino-terminated PAMAM dendrimers and PAMAM-C12-50% dendrimers, the higher the concentration of dendrimer the bigger the disturbances. On the contrary, for PAMAM-CH_3 dendrimers, we observed that increasing dendrimer concentration did not cause a proportional enhancement of bilayer perturbances. This would indicate that for higher dendrimer concentrations, two independent processes occurred at the same time: interactions between dendrimers and bilayer, and also creation of stable aggregated dendrimer structures. A similar situation was observed for grafted amphiphilic PVP-palmityl polymers (Savva et al., 1999). The increased concentration of this polymer above a certain level resulted in a complete recovery of the DPPC transitions, instead of progressive changes in the thermotropic behavior. In our case, DSC data indicates that the DPPC bilayer was not completely free of incorpo-

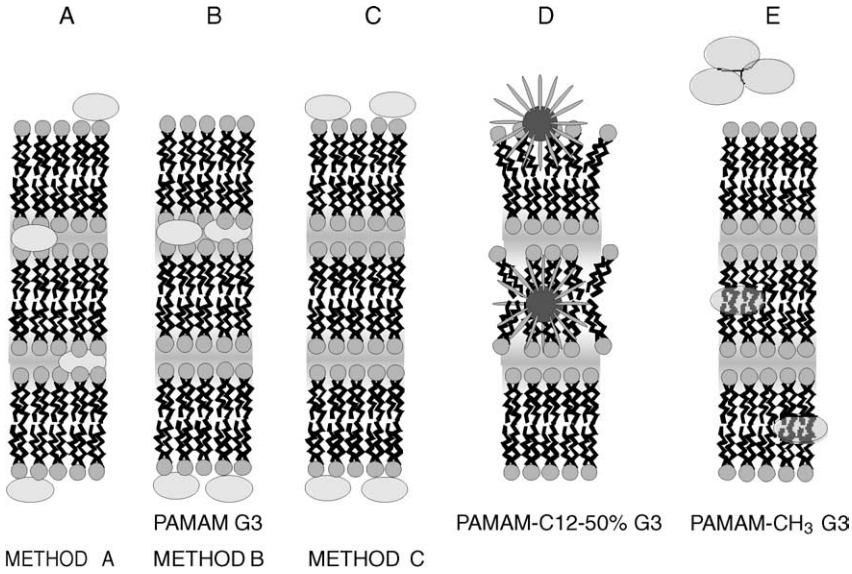

Fig. 8. Models of interactions between dendrimers and MLVs.

rated dendrimers but new molecules did not enter into liposomes.

Energy is required to convert MLVs to SUVs since the inner and outer monolayers of an SUV have to attain curvatures of opposite sign. In some cases when a "foreign" molecule is present, SUVs do not form with the input of ultrasound energy. We suggest that SUVs do not form as readily when the dendrimer is first incorporated into the lipid film. The resulting DSC curve shows a sharper peak at 24 °C (Fig. 7), as observed for MLVs. In the case of PAMAM-CH_3, the peak is asymmetric, suggesting a non-uniform distribution of dendrimer as indicated above. The greatest disturbance was observed in the presence of the PAMAM dendrimer when liposome–dendrimer solution was prepared as described in method B. The shifts in phase transition temperature in this case was greater than that observed with MLVs.

5. Conclusions

Polyamines belong to a group of substances that cannot easily penetrate the bilayer (Bertoluzza et al., 1995). Hydrophilic amino-terminated PAMAM dendrimers would be located near lipid head groups and interact with the phosphate headgroup of the lipid. When solutions were prepared according to method A, dendrimers were uniformly located between bilayers and on the surface of MLVs (Fig. 8A). When dendrimers were added at the hydration stage (method B) experimental conditions were favorable for creation of dendrimer-rich and dendrimer-poor domains (Fig. 8B). This resulted in a larger thermotropic disturbance. Adding dendrimers after MLVs were prepared allowed them to locate only near the outer surface (Fig. 8C).

Both PAMAM-CH_3 and PAMAM-C12-50% dendrimers are not soluble in water and therefore interacted in a different way with a bilayer. The presence of long chains on the surface of PAMAM-C12-50% seems to be responsible for the disruption of the bilayer (Fig. 8D). PAMAM-CH_3 dendrimers induced smaller changes in the bilayer structure. They are flat, ellipsoid molecules and probably they could accommodate in the hydrophobic part of bilayer without causing loss of integrity (Fig. 8E). When the dendrimer concentration was high, stable dendrimer aggregates were created which could not easily be incorporated into the bilayer.

To summarize, the two important factors that determine the extent of interactions of dendrimers with membranes are their relative hydrophobic/hydrophilic affinity with portions of the bilayer and their intrinsic shape. It would be useful to study further details of the structural and dynamic properties of the complexes of dendrimers with bilayers using NMR and diffraction methodologies.

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