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Interaction of cationic partial dendrimers with charged and neutral liposomes☆

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Abstract

Amphipathic partial dendrimers having three lipidic (C_{14}) chains coupled to dendritic lysine head groups with eight, 16 or 32 free terminal amino groups have been synthesised by solid-phase peptide synthesis. Liposomes were prepared with positive, negative and neutral charge using the dehydration–rehydration method and their interaction with the partial dendrimers studied. The interaction efficiency of the partial cationic dendrimers studied was greater than 88%. Interaction of the cationic partial dendrimer converted liposomes with very low or negative charge into positively charged species. Apparent vesicle size increased with the head size of the partial dendrimer but, in the case of negatively charged liposomes, large changes in properties were observed after ultracentrifugation due to the formation of myelin figures. To investigate the mode of interaction with the liposomes, adsorption studies were performed by adding the partial dendrimer after the preparation of dehydration–rehydration vesicles. The results indicated that adsorption is inversely proportional to the head size of the partial dendrimer molecules and the extent of adsorption was similar on both positively and negatively charged liposomes. Adsorption produced liposomes with greater or similar zeta potentials to liposomes that incorporated partial dendrimer through the dehydration–rehydration method. Taking account of the different interaction efficiencies, this suggests there is a degree of partial dendrimer entrappment inside the liposomes.

Keywords: Dendrimers; Lipidic peptide dendrimer; Liposomes; Liposome–dendrimer interactions; Adsorption

1. Introduction

Dendrimers are highly branched and reactive three-dimensional polymers, with all bonds emanating from a central core. Since their introduction in the mid-1980s, this novel class of polymeric materials has attracted considerable attention because of their unique structure and properties. Compared with traditional linear poly-

Abbreviations: DSPC, distearoyl phosphatidylcholine; CHOL, cholesterol; PG, phosphatidyl glycerol; DC-CHOL, dimethylaminoethyl carbamate cholesterol; DRV, dehydration–rehydration vesicle.

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mers, dendrimers have much more accurately controlled structures, with a generally globular shape, a single molecular weight rather than a distribution of molecular weights, and a large number of controllable peripheral functionalities (Tomalia et al., 1990). Since dendrimers have a symmetrical quasi-spherical or spherical topology, the dendrimers described in this communication can be termed 'partial dendrimers' or 'dendrons' because they are asymmetrical, having a lysine head group coupled to a complex lipophilic tail.

Several categories of dendrimers have been synthesised with various surface functional groups (Denkalwalter et al., 1981; Tomalia et al., 1990; Uhrich et al., 1991; Shao and Tam 1995; Sakthivel et al., 1998; Liu and Fréchet 1999). Potential uses include molecular transport vehicles, molecular ball bearings, flow regulators in fluids, diagnostic products, artificial enzymes and vaccines (Dvornic and Tomalia, 1996; Liu and Fréchet, 1999). Lipid-modified peptide dendrimeric adjuvants have been employed to increase the immunogenicity of synthetic peptides (Toth et al., 1993; Toth, 1994). Peptide-based dendrimer systems with cationic surfaces are under investigation as gene delivery vectors in our laboratories (Toth et al., 1999). Attempts have been made to conjugate model drugs such as amino acid derivatives and cholesterol to water-soluble dendrimer–polyethylene glycol (Liu et al., 1999).

Although many different types of dendrimers have been synthesised, interaction between charged dendrimers and vesicular structures does not seem to have been investigated. We have previously reported the synthesis of dendrimers and partial dendrimers with lipid character, free amino groups and amphiphilic properties (Sakthivel et al., 1998; Toth et al., 1999). Oral uptake of one of the lipid dendrimers has been studied elsewhere (Florence et al., 2000). In this paper, we report the synthesis of radiolabelled partial dendrimers and interaction of these partial dendrimers with charged and neutral liposomes. These partial dendrimers, although soluble in water, have three lipidic chains to improve transmembrane transport potential. They can be either entrapped inside the aqueous phase of the liposomes, entrapped in the lipid bilayer, adsorbed onto the surface or co-exist between two of the states mentioned.

2. Synthesis of the partial dendrimers

The synthesis of the series of lipidic peptide partial dendrimers has been described in detail elsewhere (Sakthivel et al., 1998). Briefly, they are

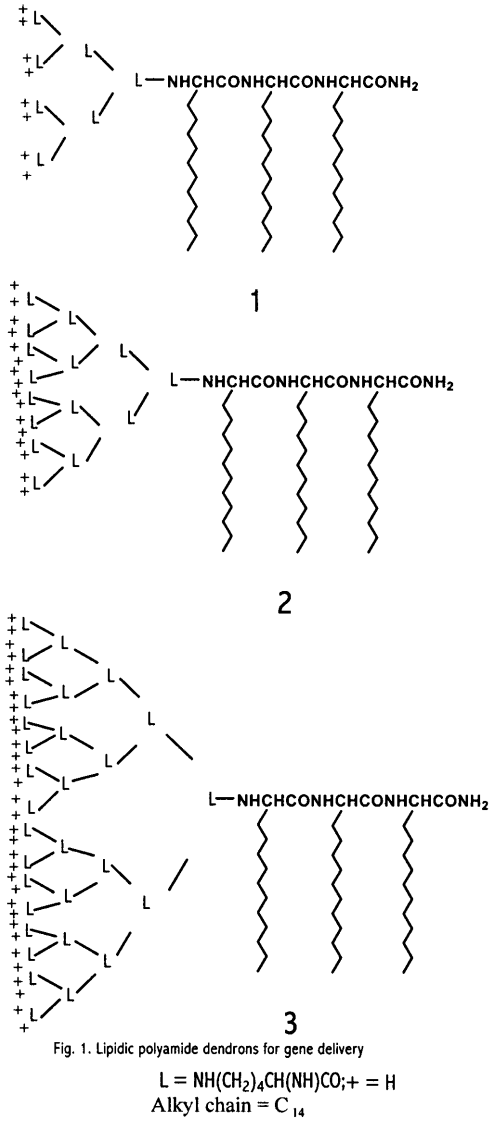

Fig. 1. Lipidic polyamide dendrons for gene delivery

Fig. 1. Schematic diagram of the cationic partial dendrimers studied.

synthesised from appropriately protected lysine, and 2-amino-tetradecanoic acid was prepared by solid-phase peptide synthetic methods (Fig. 1). To introduce radiolabelling, tritiated lysine was converted to Boc-Lys(Boc)-OH using standard methods. During synthesis, initially tritiated protected lysine was reacted with the resin, and the reaction was subsequently completed with unradiolabelled protected lysine. The dendrimers were cleaved by the HF method, purified by high-performance liquid chromatography and their molecular weight confirmed by matrix-assisted laser desorption ionisation mass spectrometry.

3. Preparation of liposomes

The lipids used for the preparation of liposomes were obtained from Sigma Chemical Company (UK). Liposomes with different compositions containing distearoyl phosphatidylcholine (DSPC)/cholesterol (CHOL) (1:1, neutral), DSPC/CHOL/phosphatidyl glycerol (PG) (1:1:0.1, negatively charged), and DSPC/CHOL/dimethylaminoethyl carbamate cholesterol (DC-CHOL) (1:1:0.1, positively charged) were prepared according to the dehydration–rehydration method (Gregoriadis 1998). After initial hydration of lipid mixture, radiolabelled dendrimer (2 mg) solution was added, freeze-dried and rehydrated. The suspension was ultracentrifuged at 25 000 rpm for 30 min, washed to remove any unentrapped/non-interacted dendrimer and recentrifuged. The pellet was suspended in 6 ml water and used for further studies. The radioactivity of the dendrimers were measured by using the scintillation counter to determine the encapsulation efficiency.

4. Measurement of vesicle size and zeta potential

Vesicle size was measured using a Mastersizer X (Malvern Instruments, Malvern, UK). To measure the zeta potential, 20 μl was diluted to 10 ml with double-deionised water. Samples were placed and analysed immediately in a Zetasizer 3000 (Malvern Instruments) with a He–Ne laser; the angle of measurement was 90°. Five repeat measurements were carried out at 30 s intervals to equilibrate the samples.

5. Adsorption studies

To determine the amount of partial dendrimer associated with the surface of liposomes, empty liposomes were prepared by the dehydration–rehydration vesicle (DRV) method as already described and quantities of radiolabelled dendrimer (0.33 mg) were added to 1 ml liposome (DRV) suspension, mixed and left for 5 h at 4°C. The resultant liposomes were ultracentrifuged at 25 000 rpm for 30 min and the radioactivity associated with the liposomes from the resuspended pellet was measured.

6. Results and discussion

The structures of the partial dendrimers with 8–32 amino groups are presented in Fig. 1. The structures were confirmed by mass spectrometry. The entrapment/interaction efficiency of the three dendrimers studied ranged from 88 to 98%. The interaction studies carried out between dendrimers and liposomes were performed with three types of liposomes, namely neutral, negative and positive.

Negatively charged liposomes formulated with DSPC/CHOL/PG showed large increases in apparent diameter with increasing number of amino groups of the partial dendrimers used (see Fig. 2). This graph represents the vesicle diameter following ultracentrifugation to remove non-interacted partial dendrimer. The photomicrographs of Fig. 3a–c show negatively charged liposomes, with and without the 32 amino group partial dendrimer present. Fig. 3c shows the formation of large vesicular structures (average apparent diameter, 47.4 μm) with myelin figures, following ultracentrifugation. This suggests that the amphipathic partial dendrimers solubilise the liposomes. While similar results were obtained using eight and 16 amino group partial dendrimers with negatively charged liposomes, but to a lesser extent, the mechanism involved is still unclear.

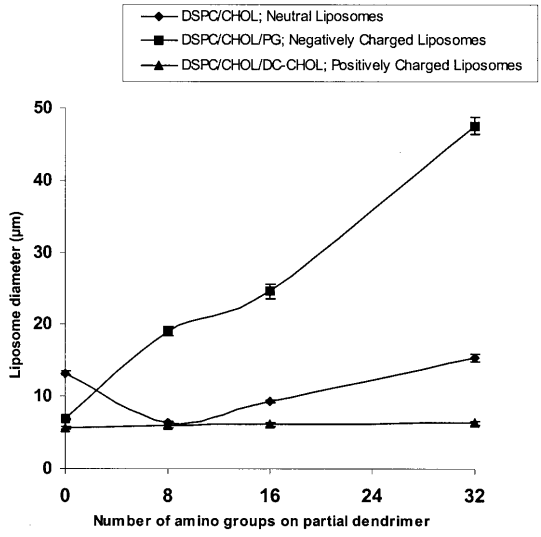

Fig. 2. Effect of dendrimer interaction on apparent vesicle diameter.

The effect of the cationic partial dendrimers on the zeta potential of the liposomes is presented in Fig. 4. The zeta potentials of the control liposomes (without additive) were −42 mV for the negatively charged liposomes and 26 mV for the positively charged liposomes. When the cationic partial dendrimers are present, all three formulations of liposomes produced positively charged species ranging from 35 to 61 mV.

The extent of interaction of the partial dendrimers studied with liposomes were all in the range 88–98%. The head size of the partial dendrimers did not appear to affect the interaction efficiency. To investigate the mode of interaction, placebo liposomes were prepared by the DRV method (Gregoriadis 1998) and radiolabelled partial dendrimers were added externally, mixed, left for 5 h and ultracentrifuged at 25 000 rpm. The effect of adsorption of partial dendrimers to liposomes is presented in Fig. 5, indicating that partial dendrimer adsorption is clearly dependent on head size and not charge on the liposome. Partial dendrimer adsorption was calculated from the amount of partial dendrimer remaining, after ultracentrifugation, as a percentage of the initial amount added. Tables 1 and 2 show the amount of partial dendrimer present and the resultant zeta potential for both interaction/entrapment and adsorption investigations, respectively, for the liposome formulations used. The amount of partial

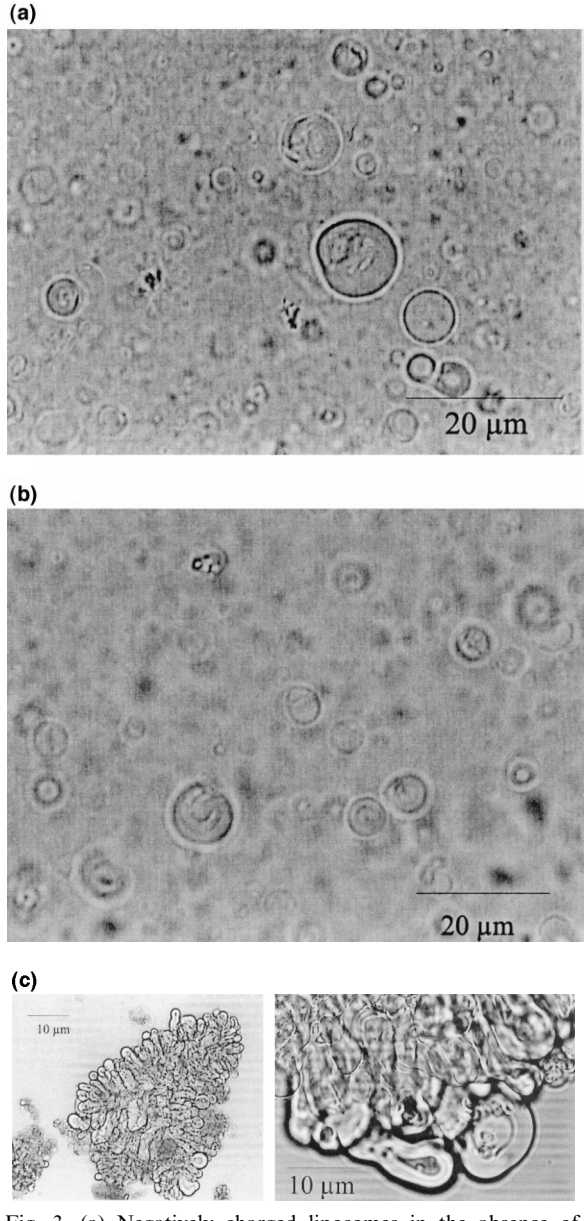

Fig. 3. (a) Negatively charged liposomes in the absence of partial dendrimer. (b) Negatively charged liposomes in the presence of the 32 amino group partial dendrimer before ultracentrifugation (during interaction/entrapment investigation). (c) Negatively charged liposomes in the presence of the 32 amino group partial dendrimer after ultracentrifugation (during interaction/entrapment investigation).

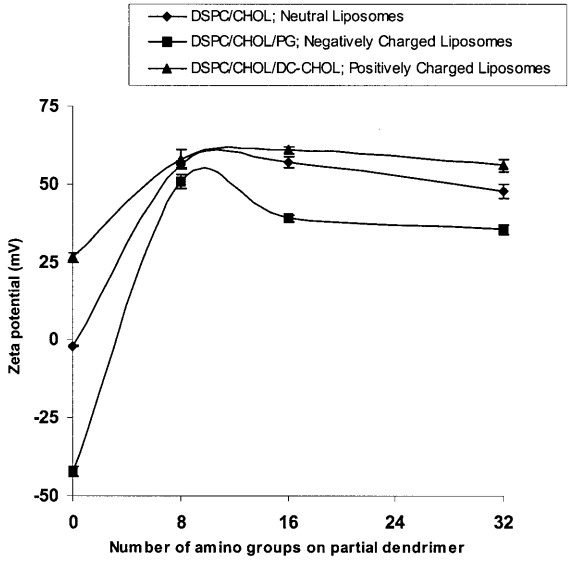

Fig. 4. Effect of partial dendrimer interaction on zeta potential.

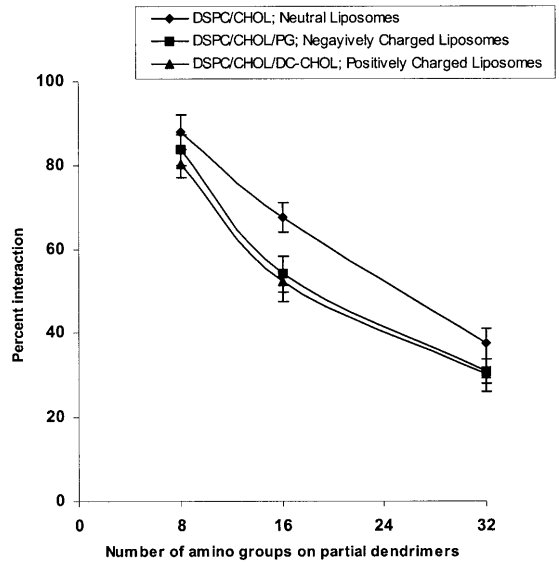

Fig. 5. Adsorption of partial dendrimer on liposomes.

dendrimer adsorbed decreases with increasing partial dendrimer head size. This ranges from approximately 80% adsorption for the compound with eight amino groups to approximately 30% adsorption for those with 32 amino groups; this trend is evident for all three liposome formulations. The zeta potential of the adsorbing liposomes is similar (between 56 and 68 mV). This order of effect cannot be seen with the interacting/entrapping liposomes. With regard to greater interaction of the partial dendrimer with liposomes, zeta potentials are similar or lower (between 35 and 58 mV) than those of the adsorbing liposomes. This suggests that there is a degree of interaction in the interior of the liposomes.

The mechanisms by which the cationic partial dendrimers interact with vesicles are still unclear, but their charged amphipathic structure makes a variety of mechanisms possible. Although cationic, these partial dendrimers do not show preference for anionic liposomes and adsorb to all liposomes similarly, suggesting hydrophobic interaction.

Table 1
Interaction/entrapment investigation results showing the amount of each partial dendrimer present and the resultant zeta potential for neutral (DSPC/CHOL), negatively charged (DSPC/CHOL/PG) and positively charged (DSPC/CHOL/DC-CHOL) liposomes

Initial charge of liposome	Number of amino groups on partial dendrimer	Molecular weight of partial dendrimer	% of partial dendrimer interacting/entrapped	Average zeta potential (mV)
Neutral	8	1587.30	98.01	56
	16	2612.06	90.36	57
	32	4661.58	89.83	48
Negative	8	1587.30	97.50	51
	16	2612.06	88.04	39
	32	4661.58	93.99	35
Positive	8	1587.30	96.47	58
	16	2612.06	88.97	61
	32	4661.58	95.09	56

Table 2
Adsorption investigation results showing the amount of each partial dendrimer present and the resultant zeta potential for neutral (DSPC/CHOL), negatively charged (DSPC/CHOL/PG) and positively charged (DSPC/CHOL/DC-CHOL) liposomes

Initial charge of liposome	Number of amino groups on partial dendrimer	Molecular weight of partial dendrimer	% of partial dendrimer adsorbed onto liposome surface	Average zeta potential (mV)
Neutral	8	1587.30	87.95	68
	16	2612.06	67.54	68
	32	4661.58	37.46	67
Negative	8	1587.30	83.71	63
	16	2612.06	53.99	60
	32	4661.58	30.89	61
Positive	8	1587.30	80.23	56
	16	2612.06	52.13	58
	32	4661.58	30.10	61

Acknowledgements

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