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Note

Dendrisomes: cationic lipidic dendron vesicular assemblies

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Abstract

A new lipidic cationic polylysine dendron was prepared by solid-phase peptide synthesis. Its behaviour in aqueous media and its ability, with and without cholesterol, to form higher order structures, "dendrisomes", was studied to further our understanding of how dendrons interact with drug molecules and may be utilised as drug carriers. Dynamics simulations of the dendron show their flexibility. Incorporation of cholesterol increases the hydrodynamic diameter of the aggregates from 311 to 556 nm but does not affect their positive zeta potential (of the order of +50 mV). The dendrisomes encapsulated penicillin G (6.15% w/w) compared to only 1.4% w/w entrapment in REV liposomes of 1:1 distearoyl phosphatidylcholine:cholesterol. Cholesterol, however, decreases the entrapment efficiency. Electrostatic forces and H-bonding between the negatively charged drug and dendron amino groups are likely to be key in determining these interactions.

Keywords: Dendrimer; Dendron; Self-assembly; Dendrisome; Drug–carrier interactions

Three dimensional hyper-branched macromolecules, commonly known as dendrimers have been known for some time (Tomalia et al., 1990) and suggested for several applications ranging from vectors for drug and gene delivery, "molecular boxes", and vaccine adjuvants (Toth et al., 1993; Liu and Frechet, 1999; Shah et al., 2000).

Engineering dendrimers and partial dendrimers or dendrons (dendrimers having no branching core) is made possible by controlling the branching units, generation number and surface functionalities. Furthermore, dendrimers and dendrons of the appropriate hydrophile–lipophile balance (HLB), size and topology self-assemble into higher order structures, broadening their potential as drug carriers (Zimmerman et al., 1996; Schenning et al., 1998; Sakthivel et al., 1998). There is little information in the literature on the use of dendrimers as drug delivery carriers. Studies on the interactions of dendrimers and drugs such as piroxicam (Wiwattanapatapee et al., 1999), 5-flurouracil (Khopade et al., 1999), ibuprofen (Milhem et al., 2000) and indomethacin (Liu et al., 2000) have, however, been published.

The present work reports on a novel cationic lipidic polylysine dendron (Fig. 1) which self-assembles in water, forming unusual small vesicular structures, which we term "dendrisomes", capable of interacting with benzyl penicillin (penicillin G) used here as a model of an orally labile, negatively charged water soluble antibiotic.

The dendron was prepared by stepwise solid-phase peptide synthesis, from Boc-Lys(Boc)-OH and 2-amino tetradecanoic acid, on 4-methyl benzhydrylamine (MBHA) resin using BOC-strategy as described elsewhere (Al-Jamal et al., unpublished data; Sakthivel et al., 1998). The molecular weight (3388) was con-

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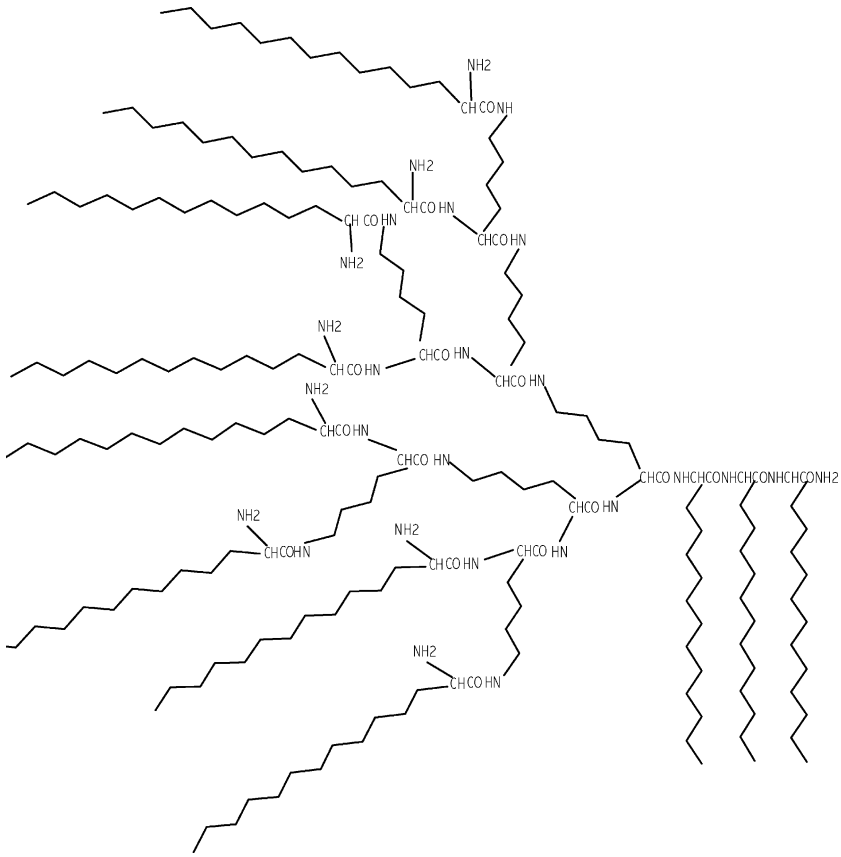

Fig. 1. The chemical structure of the lipid-lysine dendron synthesised.

firmed by FAB-MS. Dynamics simulations of the dendron were performed over 40 ps in vacuum, and with a distance dependent dielectric to mimic the effect of a polar environment using (QUANTA/CHARMm) and Sybyl. Furthermore, to mimic a water/air interface, the dendron was placed in close proximity to the surface of a slab of water molecules. Water molecules had fixed atom constraints applied throughout the simulation.

Distearoyl phosphatidylcholine (DSPC) and cholesterol (CHOL) was obtained from Sigma Chemical Company (UK). Different dendron/CHOL molar ratios (1:0, 1:1, 1:5, 1:7, 1:9) were prepared according to Reverse-Phase Evaporation method. Twenty milligrams of the total lipid (dendron/CHOL) were dissolved in 40 ml chloroform:ether (1:1) mixture, 5 ml of deionised water or radiolabelled penicillin G in solution was injected into the organic solution, bath sonicated for 2 min, and the organic solvent removed under reduced pressure. The dispersion was bath-sonicated at 65 °C for 2 h. Liposomes containing DSPC:CHOL (1:1) were prepared using the same technique. The suspension was ultra-centrifuged at 40,000 rpm for 1 h, washed to remove any unentrapped/non-interacting drug. The pellet was suspended in 1 ml. The radioactivity of the drug was measured to determine the encapsulation efficiency. The supramolecular aggregates (dendrisomes) were examined by Philips CM 120 Bio Twin (Eindhoven, Holland) transmission electron microscope, samples being negatively stained with 1% uranyl acetate. Hydrodynamic Z-average sizes and zeta potentials were measured using a Zetasizer 3000 (Malvern Instruments, Malvern, UK).

The dendron was synthesised to alter the lipophilicity of a previously synthesised dendron (Shah et al., 2000), to determine how lipophilicity and geometry can affect the process of self-assembly, drug interac-

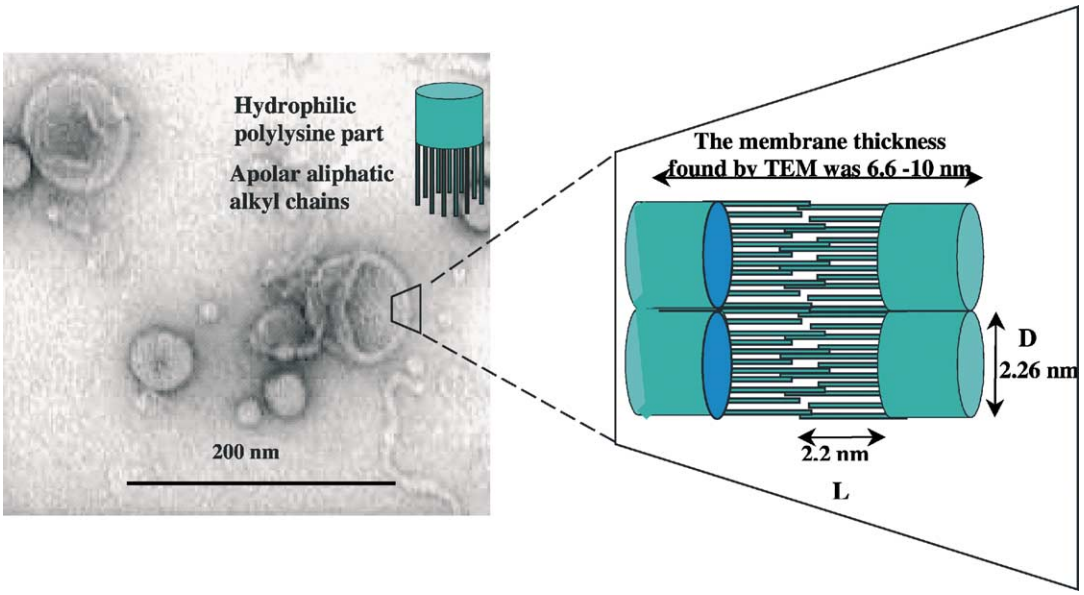

Fig. 2. Transmission Electron Micrograph of dendrisomes with membranes from 6 to 10 nm thick (left). Schematic representation of bilayer formation (right). The diameter, *D*, was calculated from the molecular area found by surface pressure studies. The length of the alkyl chain, *L*, based on Corey–Pauling–Koltun (CPK) molecular modelling.

tion and possibly final destination in the body. Although lysine amino terminals were coupled to the lipoamino acid, the amino groups of the lipid remain unacetylated to impart an overall positive charge to the dendron.

The dynamics simulations "in vacuo" show that the lipidic chains display a lower tendency to aggregate. On the other hand, collapse of the lipidic chains and internalisation within the globular structure when the simulations were done with a distance-dependent dielectric mimicking the effect of a polar environment, the charged amino groups generally oriented outwards from the centre of the molecule. Experimentally, acetylation of the alkyl amino group results in a completely insoluble compound which fails to self-assemble in water. Simulation of the water/air boundary reveals that the dendron tends to align in a way that nitrogen atoms are attracted towards water, the hydrophobic part extending outwards.

The lipid-modified cationic dendron was found to self-assemble in the absence of cholesterol into vesicular structures with a Z-average diameter of 311 ± 8 nm. The zeta potential (+56 mV) was positive. TEM images (Fig. 2) show membranes of the vesicles to be from 6 to 10 nm thick. We postulate, based on calculated dimensions, the formation of bilayer structures. The polylysine head groups make the dendrisome membrane bulkier and thicker compared to liposomal membranes where the thickness is around 5 nm.

Cholesterol was found to have an effect on the morphology and size of the dendrisomes but not on their charge. Dendrisomes without cholesterol are of the order of 311 nm in diameter and have a more uniform morphology than those dendrisomes with cholesterol, which are in the formulations studied 557 ± 13 nm in diameter. The shape and irregularity of the supramolecular aggregates increases with increasing cholesterol content (to be published). On the other hand, the effect of cholesterol incorporation was less significant on the value of the zeta potential: with the highest level (i.e. dendron/cholesterol 1:9 molar ratio), the zeta potential of the dendrisomes fell only from +56 to +50 mV. Cationic dendrisomes were found to have a higher encapsulation efficiency compared to neutral REV liposomes (1.4%). Despite the fact that increasing the cholesterol percentage in the total lipid (dendron + cholesterol) increased dendrisome size (Fig. 3a), the percentage drug entrapment (mg drug

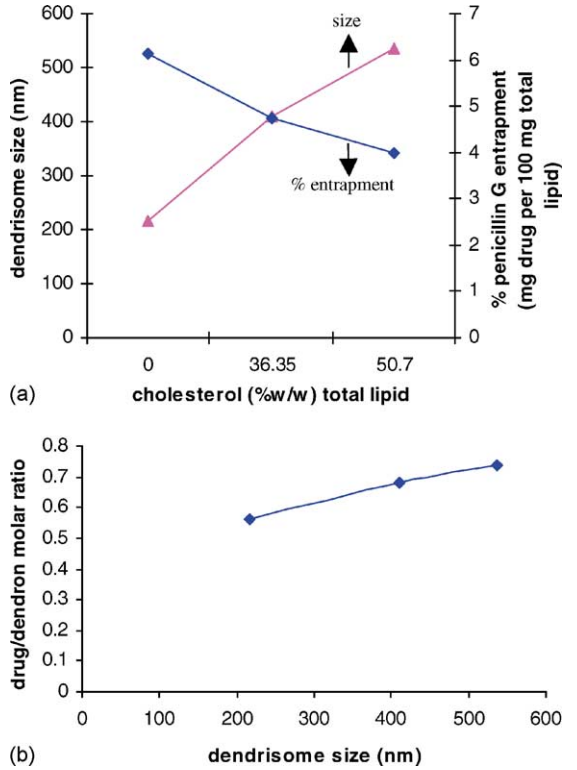

Fig. 3. Percentage drug entrapment (mg drug per 100 mg total lipid) (◆) and Z-average diameters of dendrisomes (▲) as a function of percentage cholesterol in total lipid (i.e. dendron + cholesterol). (a) The effect of cholesterol is to increase the average diameter of the dendrisomes, but reduce the percentage of penicillin G entrapment. The relationship between the Z-average diameters of dendrisomes (formed with different contents of cholesterol) and the drug/dendron ratio. (b) Dendrisomes with the largest Z-average diameter (highest cholesterol content) had the maximum drug/dendron ratio of interaction, although, as seen in (a), the percentage entrapment calculated on the basis of drug/total lipid was the least.

per 100 mg total lipid) was reduced. Cholesterol-free dendrisomes (the smallest) had the maximum entrapment efficiency (6.15%). Percentage entrapments of 4.74 and 4% in case of dendron/CHOL molar ratios 1:5 and 1:9 were achieved, respectively. This could be due to reduced drug accumulation at the dendron sites in the bilayers as a consequence of replacements of dendrons, a potential site of electrostatic interaction or H-bonding, with cholesterol. However, such interactions are not the only key in determining entrapment. It was found that (Fig. 3b) increasing the size of dendrisomes (by adding more cholesterol) increased the drug/dendron molar ratio, which suggests the involvement of other factors which need further study.

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