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Note

Adsorption of amphipathic dendrons on polystyrene nanoparticles

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Abstract

Adsorption of dendrons onto nanoparticles may provide new model structures which may be useful in drug and gene delivery. Tritiated amphipathic dendrons having three lipidic (C_{14}) chains coupled to branched (dendritic) lysine head groups with 8, 16 or 32 free terminal amino groups have been synthesised by solid phase peptide techniques. The interaction between these tritiated dendrons and 200 nm polystyrene latex nanoparticles was investigated in phosphate buffered saline. The amount of dendron adsorbed increased with increasing concentration of dendrons and then decreased. Maximum adsorption of dendrons per gram of nanoparticles was found to be between 8.2 and 84×10^{-6} M, the amounts adsorbed being inversely proportional to the number of amino groups present in the molecule. The number of dendron molecules adsorbed per nanoparticle was found to be between 430 and 4421. The degree of adsorption was found to be slightly altered by the temperature.

Keywords: Dendrimers; Dendrons; Adsorption; Polystyrene nanoparticles

Partial dendrimers or dendrons are highly branched and reactive three-dimensional molecules, with up to 32 terminal groups. This novel class of polymeric materials has attracted considerable attention because of their unique structure and properties (Sakthivel, 2002). Compared with traditional linear polymers, dendrons and 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" as they are asymmetrical, having a lysine head group coupled to a complex lipophilic tail.

Lipid modified peptide dendrimeric adjuvants have been employed to increase the immunogenicity of synthetic peptides. Peptide-based dendrimer systems with cationic surfaces are under investigation as gene delivery vectors in our laboratories (Toth et al., 1999). Recently Wimmer et al. (2002) reported the synthesis of novel polycationic lipophilic peptide cores and successful oligonucleotide transfection of human retinal pigment epithelium cells.

Goino and Esumi (1998) have studied the interactions of polyamidoamine dendrimers with alumina particles and concluded that the lower generation dendrimers operate as electrolytes and that the higher generation dendrimers behave like an anionic surfactant or polyelectrolyte for alumina dispersion. We have previously reported the interaction of partial dendrimers with charged and neutral liposomes (Purohit et al., 2001). In this communication we report their ability to adsorb onto polystyrene nanoparticles,

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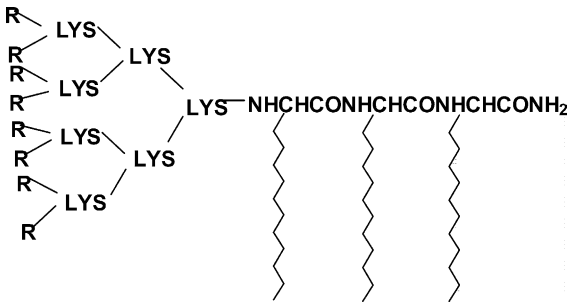

Fig. 1. Schematic diagram of dendrons studied. $(C_{14})_3$ L_7 $(NH_2)_8$ R=H, $(C_{14})_3$ L_{15} $(NH_2)_{16}$ R=LYS, $(C_{14})_3$ L_{31} $(NH_2)_{32}$ R=LYS(LYS)$_2$.

which may be useful in the design of novel materials for gene delivery.

The synthesis of a series of lipidic peptide partial dendrimers has been described in detail elsewhere (Sakthivel et al., 1998). Briefly they are synthesised from appropriately protected lysine and 2-amino tetra decanoic acid by solid phase peptide synthetic methods (Fig. 1). Partial dendrimers were cleaved by high HF, purified by HPLC and molecular weight confirmed by matrix-assisted laser desorption ionisation (MALDI) mass spectrometry.

Aqueous suspensions of mondisperse polystyrene nanoparticles (size 204 ± 6 nm) calibrated with the standards supplied by National Institute of Standards and Technology were obtained from Duke Scientific Corporation, USA, their *z*-average diameter confirmed using a Zeta Sizer (Malvern Instruments, Malvern, UK).

Varying quantities (from 0.02 to 0.2 mg) of the dendrons $(C_{14})_3$ L_7 $(NH_2)_8$, $(C_{14})_3$ L_{15} $(NH_2)_{16}$, $(C_{14})_3$ L_{31} $(NH_2)_{32}$ were mixed with a known quantity of polystyrene latex nanoparticles overnight at room temperature. Suspensions were centrifuged at 13 000 rpm for 30 min. The amount of dendron adsorbed was calculated by measuring the radioactivity (Beckmann Scintillation Counter) in the supernatant and the pellet. To study the effect of temperature, samples were stored at 5, 25, 30 and 65 °C for 24 h. Although sedimentation of aggregates of nanoparticles was observed throughout the experiments, we could not determine the size due to the destabilisation of nanoparticles by dendrons.

From the adsorption isotherms the specific surface S ($m^2\,g^{-1}$) is related to the monolayer capacity by the equation

$$S = \frac{x_m}{M} N A_m \times 10^{-20}$$

where x_m is expressed in grams of adsorbate per gram of solid, M is the molecular weight of the adsorbate, N is Avogadro's constant and A_m is the molecular cross sectional area of the adsorbate, i.e. the area which an adsorbed molecule occupies on the surface of the solid in a completed monolayer, calculated using the BET equation:

$$A_m = f\left(\frac{M}{\rho N}\right)^{2/3} \times 10^{14}$$

expressed in nm^2 where f is a packing factor, M represents the molecular weight of the adsorbate and ρ is the density of the adsorbate in the ordinary liquid or solid form.

Fig. 2 shows the relationship between the amount of dendron added and the amount of dendron adsorbed onto the latex particles. The maximum adsorption for 0.266 mg of nanoparticles ranged from 36.4 μg for dendron with 8 amino groups, followed by 17.5 μg for 16 amino groups and 8.3 μg for the dendron with 32 amino groups. The degree of adsorption was inversely proportional to size, hydrophilicity and the molecular weight of the dendron studied. All the isotherms exhibited initial increase, followed by a decline (Fig. 3).

Dendron adsorption typically increased at low concentrations with then a slight decrease with increasing concentration. Assuming monolayer adsorption of dendrons on polystyrene nanoparticles, we calculated the areas occupied by adsorbed dendrons and the specific surface area of nanoparticles (Table 1). The relationship between the specific surface and cross sectional area of the dendrons is given in Fig. 4. The areas occupied by dendrons decreased with increasing generations as their cross sectional areas increased.

Goino and Esumi (1998) reported the interaction between poly (amidoamine) dendrimers with surface carboxylic groups and alumina particles. They found that there is an increase in adsorption of dendrimers with increasing generations, the opposite of our findings, which may be due to the electrostatic attractive interactions between the negatively charged dendrimer surfaces and positively charged alumina surfaces.

From the number of nanoparticles per gram (1.91×10^{15}) and the surface area of a nanoparticle

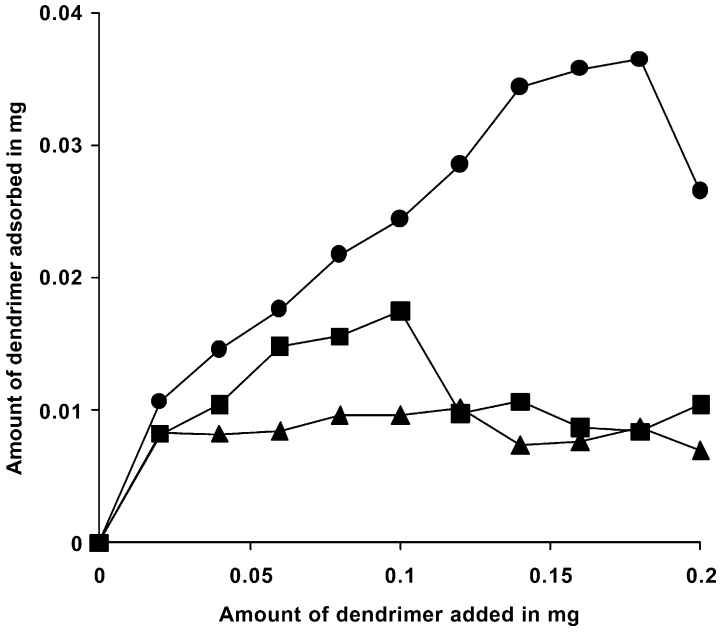

Fig. 2. Adsorption of dendrons on polystyrene nanoparticles. $(C_{14})_3$ L_7 $(NH_2)_8$ (●), $(C_{14})_3$ L_{15} $(NH_2)_{16}$ (■), $(C_{14})_3$ L_{31} $(NH_2)_{32}$ (▲).

Table 1
Data for dendron adsorption on 200 nm polystyrene nanoparticles

Dendron	Number of amino groups	MW	Maximum dendron adsorption (μM/g)	Specific surface (m^2/g)	Cross sectional area (nm^2)
1	8	1590	84.46	102.1	2.01
2	16	2615	25.08	41.1	2.72
3	32	4666	8.23	18.8	3.78

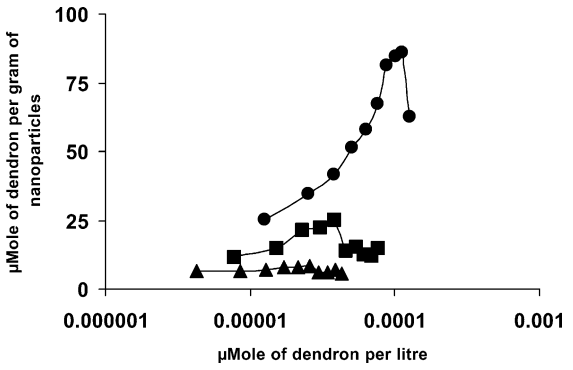

Fig. 3. Adsorption isotherms of dendrons on polystyrene nanoparticles. $(C_{14})_3$ L_7 $(NH_2)_8$ (●), $(C_{14})_3$ L_{15} $(NH_2)_{16}$ (■), $(C_{14})_3$ L_{31} $(NH_2)_{32}$ (▲).

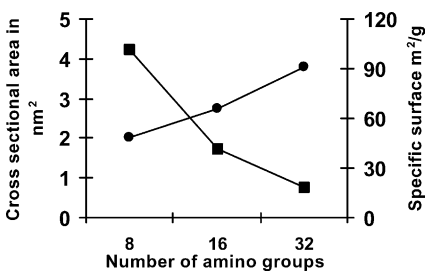

Fig. 4. The relationship between the specific surface (■) and cross sectional area (●) of the dendrons.

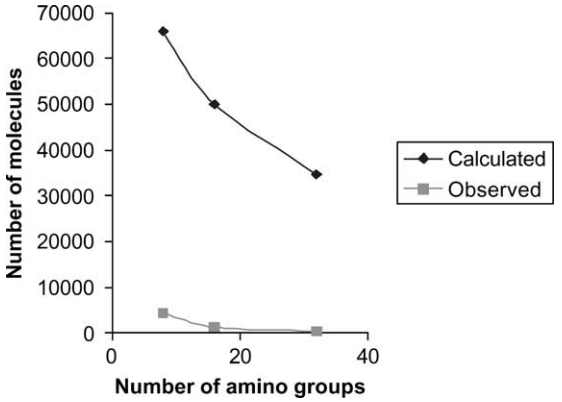

Fig. 5. The relationship between the calculated and observed number of molecules per nanoparticle.

($0.125\,\mu m^2$) the maximum number of dendrons that could be adsorbed as a monolayer on each nanoparticle (Fig. 5) ranges from 3.47 to 6.6×10^4. The maximum adsorption in reality was between 430 and 4421 molecules. Previous studies (Purohit et al., 2001) showed similar adsorption behaviour of dendrons onto liposomes.

We also studied the effect of temperature. In the case of $(C_{14})_3\ L_7\ (NH_2)_8$, increase in temperature decreased the adsorption whereas $(C_{14})_3\ L_{15}\ (NH_2)_{16}$ exhibited a slight increase, but temperature had no effect on $(C_{14})_3\ L_{31}\ (NH_2)_{32}$ adsorption.

Imae et al. (2000) concluded that the amount of adsorption is greater for the third generation dendrimer than for the fourth generation dendrimer, and that the adsorption proceeds more for surface block dendrimers than for polyamidoamine dendrimers because of their hydrophobicity. Further studies with charged nanoparticles (both cationic and anionic) will help us in understanding the mechanism of adsorption and the results may allow a better understanding of dendron–nanoparticle interactions.

Acknowledgements

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