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Note

The interaction of cationic dendrons with albumin and their diffusion through cellulose membranes

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Abstract

Amphipathic dendrons (partial dendrimers) having three lipidic (C_{14}) chains coupled to dendritic lysine head groups with 8, 16 or 32 free terminal amino groups have been synthesised by solid phase peptide synthesis. Their interaction with albumin was studied in the presence of NaCl using dynamic dialysis, the diffusion of the dendrons through regenerated cellulose membranes also being studied. The stoichiometry of dendron: albumin interactions was found to be 1:1.5, 1:4 and 1:5 for the dendrons with 8, 16 and 32 amino groups, respectively. Membrane permeability *P*, membrane diffusion coefficient *D* and the membrane partition coefficient *K* values were calculated for each dendron. *P* and *D* values were low but highest for the 8 amino group dendron. The membrane partition coefficient *K* was greatest for the 8 amino group dendron. This was also the case with octanol/water partition coefficient studies. Considerable adsorption of the dendrons to the cellulose membrane occurred but NaCl decreased adsorption and improved diffusion of the dendrons through the cellulose membrane.

Keywords: Dendrons; Partial dendrimer; Albumin; Albumin–dendron interactions; Adsorption; Diffusion; Cellulose membrane

Since their introduction in the mid-1980s, dendrimers have attracted extensive interest because of their unique structure and characteristics (Tomalia et al., 1990). Dendrimers are highly branched and reactive three-dimensional polymers, with all bonds originating from a central core. Compared with conventional linear polymers, dendrimers have much more precisely controlled structures, with a generally globular construct, a single molecular weight rather than a distribution of molecular weights, and a large number of controllable peripheral terminal groups (Tomalia et al., 1990). Since dendrimers have a symmetrical quasi-spherical or spherical topology the molecules described in this communication can be termed “partial dendrimers” or “dendrons” as they are asymmetrical, having multiple lysine head groups coupled to a relatively complex lipophilic tail.

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). Although many different types of dendrimers have been synthesised, interaction between dendrimers and proteins does not seem to have been investigated. We have previously investigated the interaction of dendrons with charged and neutral liposomes (Purohit et al., 2001). Oral uptake of one of the lipid dendrimers has also been

Abbreviations: BSA, bovine serum albumin

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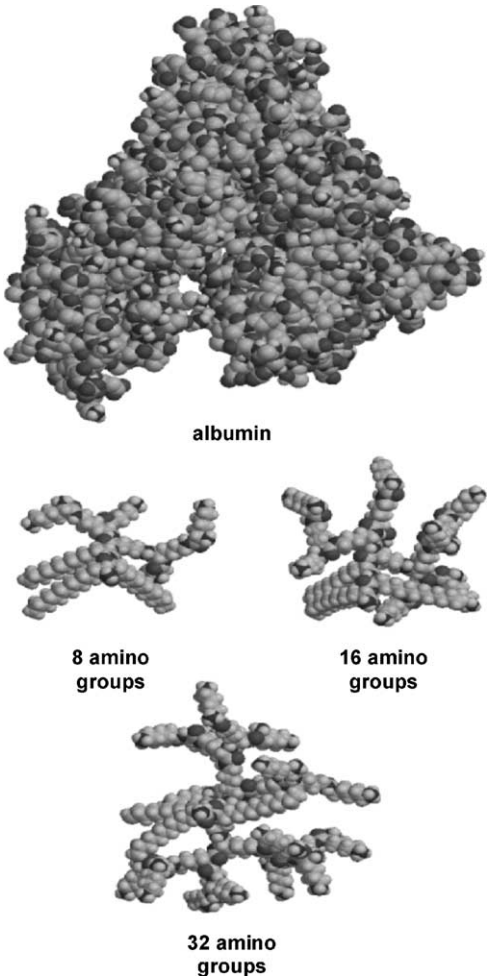

Fig. 1. Scale molecular models of albumin in comparison with 8, 16, and 32 amino group dendrons.

studied (Florence et al., 2000). Here we report the synthesis of radiolabelled dendrons, their interactions with bovine serum albumin (BSA) and their diffusion through cellulose membranes. Albumin is the most abundant protein in mammalian systems and plays an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood. The dendrons studied here (Fig. 1), although water-soluble, have three lipidic chains to improve their biomembrane transport capabilities.

BSA (Sigma, UK) was 99% fraction V, molecular mass about 66 kDa. Phosphate buffer (pH 7.4) was prepared from PBS tablets obtained from ICN Biomedicals Inc. (USA). All other chemicals were of analytical grade.

The synthesis of the series of lipidic dendrons has been described in detail elsewhere (Sakthivel et al., 1998). Briefly they are synthesised from appropriately protected lysine and 2-amino tetra decanoic acid and prepared by solid phase peptide synthetic methods.

To quantify interaction between the dendrons and albumin, dynamic dialysis (Meyer and Guttman, 1968) was performed. Aliquots (5 ml) of dendron-albumin mixtures at various molar ratios were placed in regenerated cellulose membrane dialysis tubing (SpectraPor, Spectrum Labs, Inc., USA with MWt cut off of 10 and 25 kDa), and washed before use. The tubing was sealed using magnetic weighted closures and dialysed against 500 ml of buffer at 25 °C up to 24 h in the presence of NaCl at different concentrations. Dendron interaction with the albumin and also with the cellulose membrane and the transport of dendron across the dialysis tubing was quantified by scintillation counting (LS6500, Beckman Instruments, USA). The data obtained from monitoring the transport of dendrons through the cellulose membrane was used to calculate the membrane permeability (P), membrane diffusion coefficient (D) and membrane partition coefficient (K) values of the dendrons.

The molecular masses of the partial dendrimers ranged from 1.5 to 4.7 kDa, thus all experiments were performed using dialysis tubing with a molecular weight cut off of 10 kDa, preventing the passage of the 66 kDa albumin. The diffusion data in the absence of albumin was used to calculate P, D and K values and adsorption to the dialysis membrane was also monitored. Fig. 2 represents the results after 24 h dialysis. The percentage diffusing, as expected, decreased with an increase in molecular size and number of amino groups. Adsorption complicates interpretation, as approximately 50% of each dendron adsorbs to the cellulose membrane. Fig. 3 represents the corresponding release data for the three dendrons from the dialysis tubing in the absence of albumin. The use of a membrane that had a 25 kDa cut off and was free from impurities did not significantly alter these results. The high cationic charge of the dendrons accompanied by the complex cellulose membrane structure and thickness (40 μm) hinders movement through the dialysis membrane but adsorption plays a significant retarding effect.

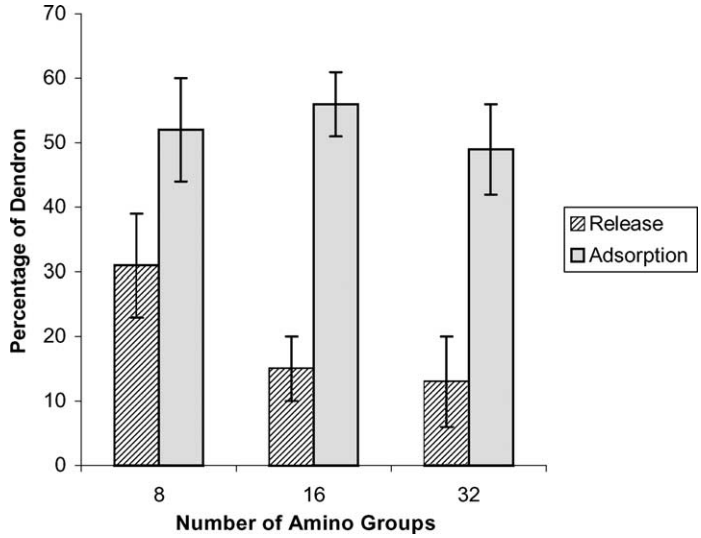

Fig. 2. Results representing percentage release of the dendrons from the dialysis tubing in the absence of albumin and the percentages of each dendron which were adsorbed to the dialysis tubing after 24 h at 25 °C.

Increasing NaCl concentrations decreased adsorption to the membrane and improved transport across the membrane with 0.8 M NaCl eliminating adsorption for all three dendrons.

Using these data, physical parameters of the membrane and an adaptation of Fick's first law, membrane permeability *P*, membrane diffusion coefficient *D* and the membrane partition coefficient *K* were calculated for each dendron. Fig. 4 shows the *P* and *D* values for the three dendrons. For the dendrons studied, the diffusion coefficient and permeability are very low through the cellulose membrane and decrease with the increasing size and number of amino groups. This can be attributed to the cationic lipidic nature of the dendrons combined with the complex structure of the cellulose membrane. The three dendrons have membrane

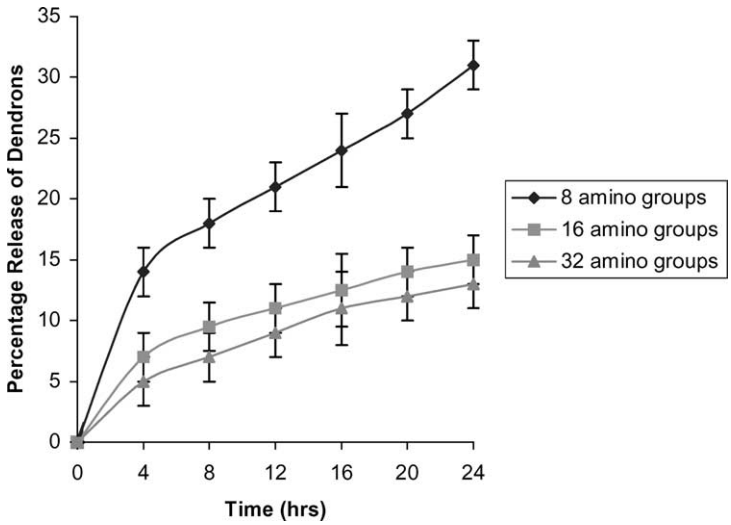

Fig. 3. Percentage diffusion profiles of the three dendrons over 24 h in the absence of albumin at 25 °C.

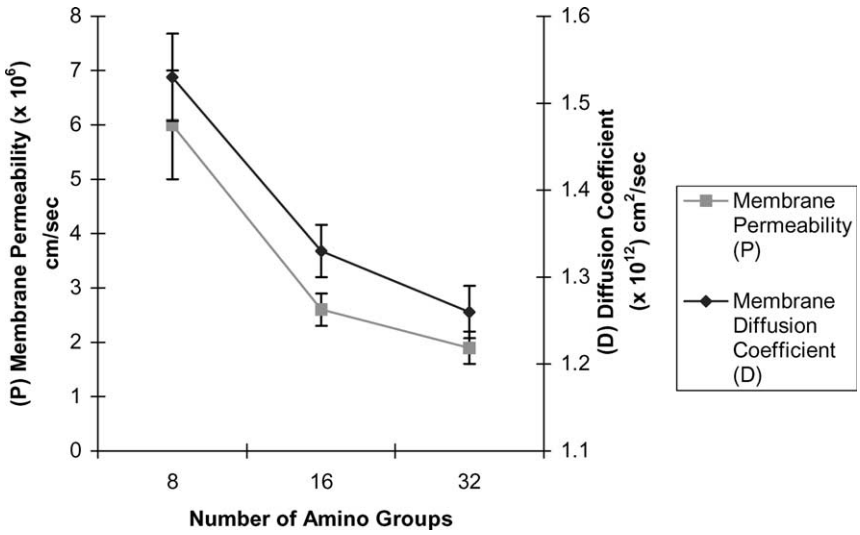

Fig. 4. Membrane permeability and diffusion coefficients for the three dendrons which were calculated using the physical parameters of the dialysis membrane.

partition coefficients (K), defined as $C_{membrane}/C_{water}$, suggesting an affinity for the cellulose membrane; the smallest dendron has a value of K of *1330*—more than twice that of dendrons with 16 (*680*) or 32 (*500*) amino groups. Log P values, calculated from measured octanol/water partition coefficients, are −0.423, −0.569 and −0.618, respectively for 8, 16 and 32 amino dendrons.

As can be seen in Fig. 1 the partial dendrons are relatively large in comparison to conventional drugs that bind to albumin. Albumin has a net formal charge of −15 but has regions of high anionic (−62) and high cationic (+58) charge. Considering regions of high anionic charge on the albumin surface, electrostatic interactions were expected. The results, represented in Fig. 5, show that none of the partial dendrimers interact with albumin in a stoichiometric manner. Approximately two dendrons with 8 amino groups interacted with three albumin molecules. Dendrons with 16 and 32 amino groups interacted with approximately four and five albumin molecules, respectively. As the albumin surface also has high regions of cationic charge,

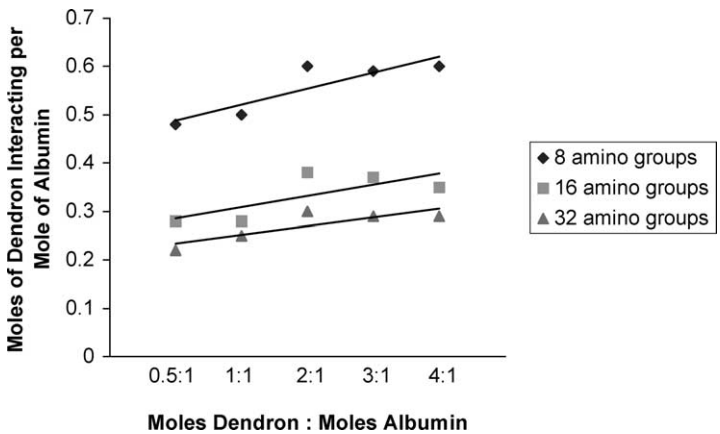

Fig. 5. Moles of dendron interacting per mole of albumin at various molar ratios after 24 h at 25 °C.

this may repel the larger more cationic molecules. The smaller dendron has a more condensed cationic charge and possibly interacts with the anionic regions of the albumin molecule.

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