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Short Communication

THE INTERACTION OF TRYPTOPHAN AND ANS WITH PAMAM DENDRIMERS

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Abstract: Dendrimers are globular, hyperbranched polymers possessing a high concentration of surface functional groups and internal cavities. These unique features make them very useful in many biomedical applications, especially as carrier molecules. In this study, the interaction of tryptophan and 1-anilinonaphthalene-8-sulfonic acid with three types of polyamidoamine dendrimers was examined. It was observed that the type of dendrimer surface group has a strong impact on the interactions between the dendrimers and fluorescent molecules.

Key Words: PAMAM Dendrimers, ANS, Tryptophan, Fluorescence

INTRODUCTION

Dendrimers are a relatively new class of polymeric material. They were discovered in the early 1980's by Donald Tomalia and co-workers [1]. Dendrimers have a specific, well-defined structure. They are synthesised from a polyfunctional core by adding branched monomers that react with the functional groups of the core molecule, in turn leaving end groups that can react again. The number of reactive terminal groups increases after the addition of the layer of monomers. The more layers of branched units that are attached, the higher the level of dendrimer generation (Fig. 1).

Because of their unique molecular architecture and globular shape, dendrimers show some interesting properties when compared to traditional linear polymers.

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Abbreviations: ANS: 1-anilinonaphthalene-8-sulfonic acid, Gn: nth generation of dendrimer, PAMAM: polyamidoamine, PBS: phosphate-buffered saline

Their many chain-ends are responsible for their high solubility and high reactivity [2]. Also, due to the presence of internal cavities, dendrimers are able to encapsulate guest molecules in their interior [3].

These specific properties make dendrimers suitable as drug delivery systems. Drug molecules can either be attached to the dendrimers' end groups, or encapsulated in the macromolecule interior. Due to the large number of terminal groups, one dendrimer molecule is capable of carrying drugs at a high density [4]. On the other hand, drugs encapsulated inside the dendrimer can be released slowly, which is an important factor in the reduction of therapeutic agent toxicity and in the avoidance of side effects [5]. Both strategies of application are very promising in targeted anti-tumour therapy.

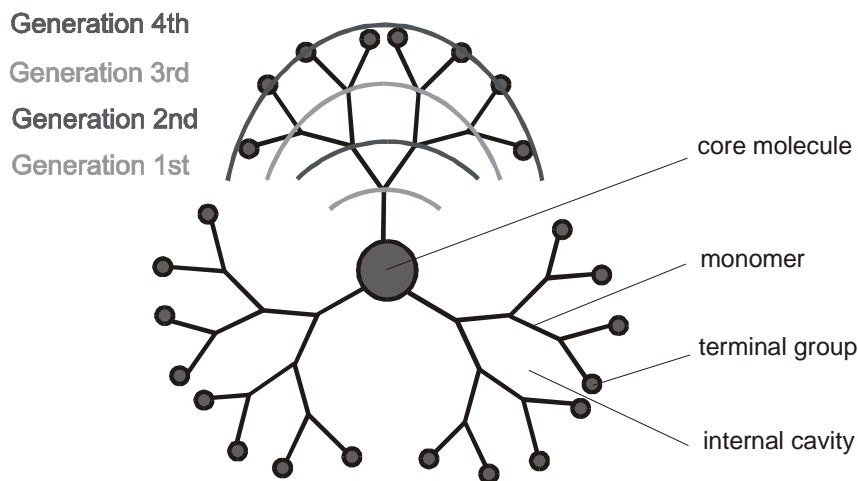
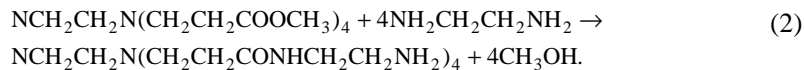
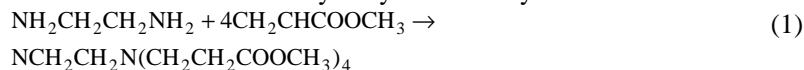


Fig. 1. A representation of a fourth generation dendrimer.

Polyamidoamine dendrimers are synthesised from an ethylenediamine core via the successive addition of methyl acrylate and ethylenediamine:



This result in each layer is being built in two steps. The half-generations of PAMAM dendrimers possess surfaces of carboxylate groups, whereas the full-generations have surfaces of amino groups. In this study, three types of PAMAM dendrimer were used – dendrimers possessing: carboxylate end groups (PAMAM G3.5); amino end groups (PAMAM G4); and hydroxy end groups

(PAMAM-OH G4). All these dendrimers have 64 end groups and a similar diameter (about 40 Å), and the molecular weight for PAMAM G3.5, PAMAM G4 and PAMAM-OH G4 equals 12419 Da, 14215 Da and 14279 Da, respectively. In this study, we examined the interaction of tryptophan and 1-anilino-naphthalene-8-sulfonic acid (ANS) with polyamidoamine (PAMAM) dendrimers. Both ANS and tryptophan fluorescence is very sensitive to changes in their environment thus these chromophores can be used to study their interactions with large dendritic macromolecules.

MATERIALS AND METHODS

Dendrimers and L-tryptophan were purchased from Aldrich (UK). 1-anilino-naphthalene-8-sulfonic acid was obtained from Sigma (USA). All the other chemicals were of analytical grade. Double-distilled water was used to prepare the solutions. Both ANS and tryptophan were dissolved in phosphate-buffered saline (PBS: 150 mmol/l NaCl, 1.9 mmol/l NaH₂PO₄, 8.1 mmol/l Na₂HPO₄, pH 7.4) at concentrations of 100 μmol/l and 50 μmol/l, respectively. Fluorescence spectra were taken with a Perkin-Elmer LS-50B spectrofluorometer at room temperature. For tryptophan fluorescence, an excitation wavelength of 280 nm was used, and the emission spectra were recorded from 300 to 440 nm. For the experiments with ANS, the excitation wavelength was set at 360 nm and the emission range was set between 400 and 700 nm. The excitation and emission slit widths were 10 nm and 2.5 nm, respectively. Samples were contained in 1 cm path length quartz cuvettes and were continuously stirred. Increasing concentrations of dendrimers were added, and the next fluorescence spectra were recorded.

RESULTS AND DISCUSSION

As tryptophan and ANS are fluorescent molecules and their fluorescence is very sensitive to changes in their microenvironment, it was possible to use spectrofluorometric methods to evaluate their interaction with dendrimers.

Changes in fluorescence intensity and a shift in the wavelength of the emission maximum upon the addition of dendrimers were registered for both tryptophan and ANS fluorescence. For tryptophan, it was evaluated as a ratio F_0/F , where F_0 and F are fluorescence intensities measured at the maximum of the emission band in the absence and in the presence of dendrimers, respectively (Fig. 2).

The basic information contained in fluorescence measurements relates to the molecular environment around the chromophore. A shift in the position of emission maximum corresponds to polarity changes in the vicinity of the chromophore molecule. Both tryptophan and ANS have higher dipole moments in the excited state than in the ground state. A more dipolar excited state interacts more strongly with solvent dipoles. Some of the energy of the excited state is then lost, and so the emission is red-shifted in a more polar solvent [6].

For example, comparing its values in dioxane and in water, the emission maximum of tryptophan shifts about 20 nm towards red [7]. The solvent shell in a nonpolar solvent is less disturbed.

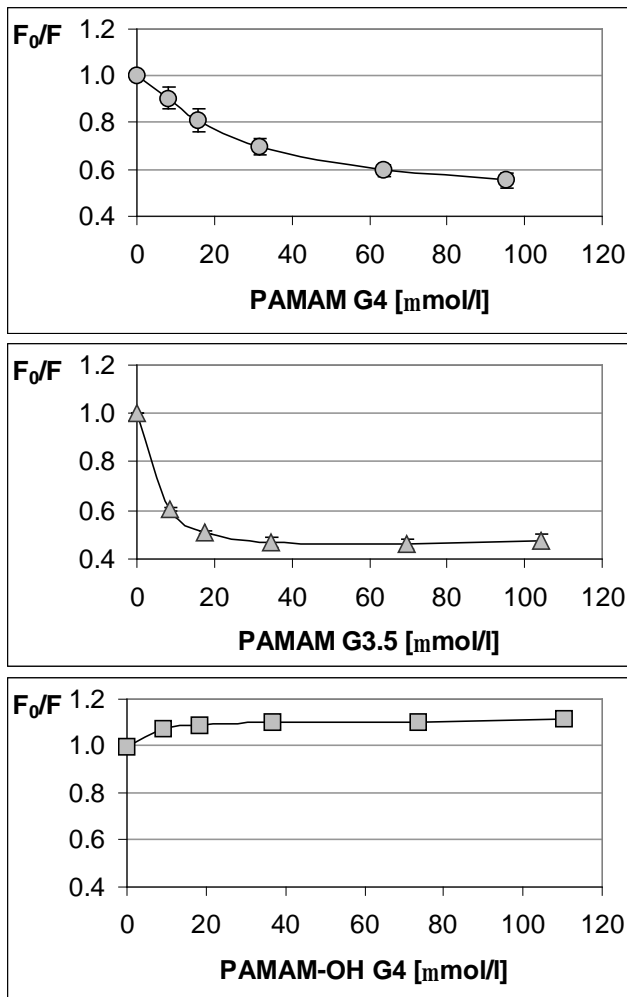


Fig. 2. The effect of dendrimers on the fluorescence intensity of tryptophan.

We observed a red shift of tryptophan fluorescence upon the addition of PAMAM dendrimers G4 and G3.5 (Fig. 3). The red shift may indicate that tryptophan molecules locate near the dendrimer surface in the region of bounded water molecules. We can assume that the excited indole ring interacts with bounded water molecules and further aligns solvent dipoles. The shift was accompanied by an increase in fluorescence intensity. Typically, the red shift of the emission maximum is accompanied by a decrease in the quantum yield of the

tryptophan. The observed increase in fluorescence intensity may be due to the effects of the probable immobilization of the indole ring. Tryptophan in neutral pH has both amino and carboxylate groups ionised. These groups can interact with charged dendrimer surface groups. Due to the lack of electrostatic interactions between tryptophan and hydroxy-terminated dendrimers, changes in the fluorescence spectra were not observed for PAMAM-OH.

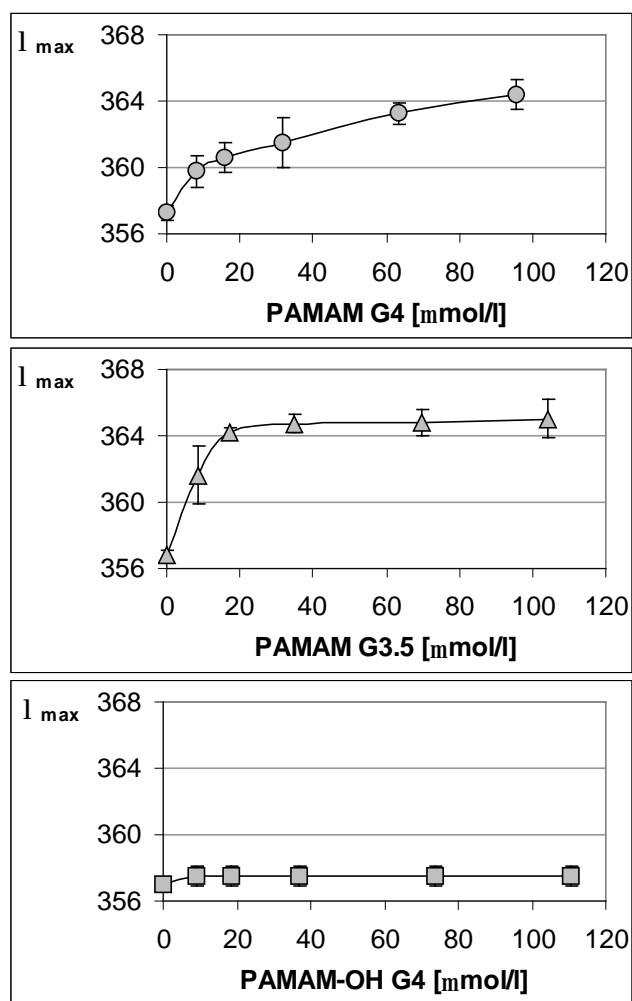


Fig. 3. The effect of dendrimers on the position of the tryptophan emission maximum.

The fluorescent dye ANS has a low fluorescent yield in polar environments, and it is greatly enhanced as the solvent polarity decreases [8]. For PAMAM G4 and PAMAM-OH G4, we observed a significant increase in ANS fluorescence (Fig. 4), whereas the wavelength of maximum emission shifted from about 516 nm to

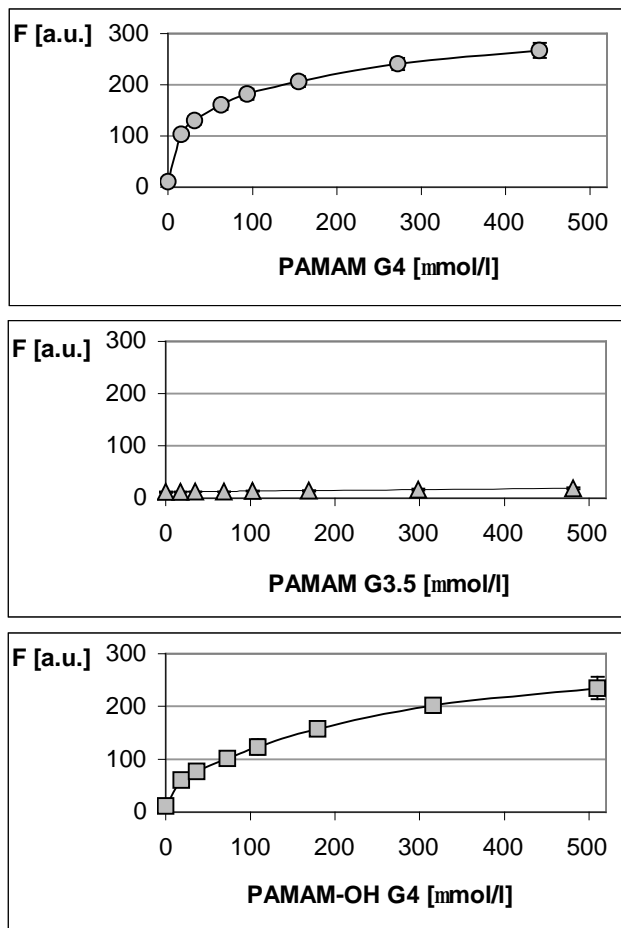


Fig. 4. The effect of dendrimers on the fluorescence intensity of ANS.

about 493 nm (Fig. 5). This suggests that ANS incorporates into dendrimer cavities, and that its aromatic rings are placed in a hydrophobic environment. No effect was observed for PAMAM G3.5 dendrimers, which possess carboxylate groups on their surface. It is very likely that electrostatic forces between their anionic carboxylate groups and the anionic sulphonate groups of ANS protect them from incorporation.

In conclusion, the results of our experiments indicate that the extent to which small molecules can penetrate into the dendrimer is under the control of the electrostatic interactions between the probe molecule and the dendrimer surface. A comparison of tryptophan and ANS behaviour revealed that not only did the molecules' charge play a great role, but also that their structure was very important. It was shown that the vicinity of the tryptophan indole ring is

hydrophilic, and is probably located on the surface of the dendrimers, whereas the ANS aromatic rings are placed more deeply in a more hydrophobic environment.

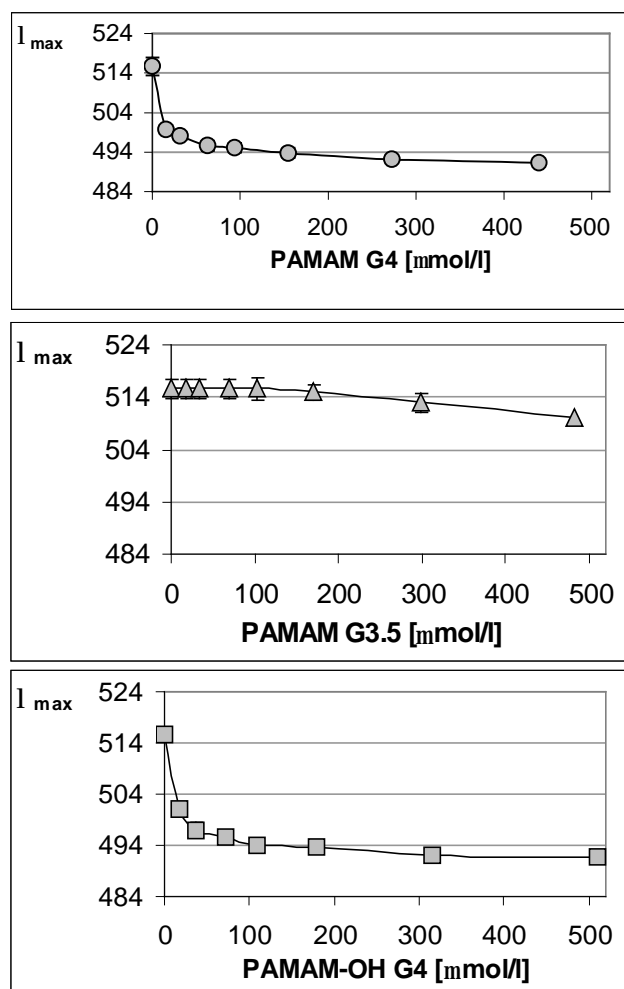


Fig. 5. The effect of dendrimers on the position of the ANS emission maximum.

REFERENCES

1. Tomalia, D.A., Baker, H., Dewald, J.R., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J. and Smith, P. A new class of polymers: Starburst-dendritic macromolecules. **Polym. J.** 17 (1985) 117-132.

2. Fréchet, J.M.J. Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. **Science** 263 (1994) 1710-1715.
3. Jansen, J.F.G.A., de Brabander van den Berg, E.M.M. and Meijer, E.W. Encapsulation of guest molecules into a dendritic box. **Science** 266 (1994) 1226-1229.
4. Zanini, D. and Roy, R. Practical synthesis of Starburst PAMAM α -thiosialodendrimers for probing multivalent carbohydrate-lectin binding properties. **J. Org. Chem.** 63 (1998) 3486-3491.
5. Kojima, C., Kono, K., Maruyama, K. and Takagishi, T. Synthesis of polyamidoamine dendrimers having poly(ethylene glycol) grafts and their ability to encapsulate anticancer drugs. **Bioconjugate Chem.** 11 (2000) 910-917.
6. Stryer, L. Fluorescence spectroscopy of proteins. **Science** 162 (1968) 526-533.
7. Ambrosone, L., D'Errico, G. and Ragone, R. Interaction of tryptophan and N-acetyltryptophanamide with dodecylpentaoxyethyleneglycol ether micelles. **Spectrochim. Acta Part A** 53 (1997) 1615-1620.
8. Slavik, J. Anilinonaphthalene sulfonate as a probe of membrane composition and function. **Biochim. Biophys. Acta** 694 (1982) 1-25.