Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 63 (2004) 193–197
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Incorporation of fluorescent probes into PAMAM dendrimers

D.M. Domański, B. Klajnert, M. Bryszewska*

Department of General Biophysics, University of Łódź, 12/16 Banacha St., Łódź 90-237, Poland

Received 23 June 2003; accepted 15 September 2003

Abstract

Interactions of two fluorescent probes 1-(trimethylammoniumphenyl)-6-phenyl-1,3,5 hexatriene *p*-toluenesulfonate (TMA-DPH) and 12-(9-anthroyloxy) stearic acid (12-AS) with polyamidoamine (PAMAM) dendrimers were studied. Changes in fluorescence intensity and steady-state fluorescence anisotropy of TMA-DPH and 12-AS were monitored. It was found that 12-AS molecules incorporated into dendrimer cavities whereas TMA-DPH molecules aggregated on the surface of polymer. Dendrimer size had not significant impact on its host properties.

Keywords: PAMAM dendrimers; Guest molecules; Fluorescence; Anisotropy; TMA-DPH; 12-AS

1. Introduction

Dendrimers have been introduced in the mid-1980s by Tomalia et al. [1]. This novel group of polymers is characterised by a specific structure. Dendrimers are globular, hyperbranched macromolecules which possess empty internal cavities and a high concentration of surface groups. Dendrimers are synthesised in a cyclic manner: branched monomers are attached to a central core molecule and then next monomers react with the outer layer of the macromolecule. The more layers of branched units are added the higher generation of dendrimer is obtained.

Polyamidoamine (PAMAM) dendrimers are based on an ethylenediamine core, and branched units are constructed from both methyl acrylate and ethylenediamine. In the current studies, we used second, third and fourth generation of PAMAM dendrimers (Fig. 1). The short characterisation of used dendrimers is presented in Table 1.

Dendrimers have attracted much interest due to the host–guest phenomena. As dendrimers are large macromolecules, it is possible to encapsulate small molecules inside them. Jansen et al. [2] trapped rose bengal and *p*-nitrobenzoic acid inside ‘dendritic box’ of polypropylene imine dendrimers. This specific property makes dendrimer suitable for a variety of biomedical applications. Among them the use of dendrimers as drug carriers has been of great interest [3]. Drugs entrapped inside the dendrimer can be released slower. The slow release is especially important for antitumour therapy because it reduces its toxicity.

The success of entrapping small molecules inside the dendrimer depends on the mutual properties between the host and the guest molecules. In the present study, the interactions of two fluorescent probes 1-(trimethylammoniumphenyl)-6-phenyl-1,3,5 hexatriene *p*-toluenesulfonate (TMA-DPH) and 12-(9-anthroyloxy) stearic acid (12-AS) with PAMAM dendrimers were examined.

2. Experimental

Polyamidoamine dendrimers (generation second, third and fourth) were purchased from Aldrich (UK). Fluorescent dyes TMA-DPH and 12-AS were obtained from Sigma (USA). Fluorescent probes at a concentration of 1 μmol/l were incubated with the following PAMAM dendrimer concentrations: 0.01, 0.1, 1, 10, 100 and 1000 μmol/l. Fluorescent measurements were carried out with a Perkin-Elmer LS-50B spectrofluorometer. The excitation and emission wavelengths were 358 and 428 nm for TMA-DPH; 360 and 471 nm for 12-AS. The excitation and emission slit widths were set to 10 and 5 nm, respectively.

* Corresponding author. Tel./fax: +48-42-635-44-74.
E-mail address: marbrys@biol.uni.lodz.pl (M. Bryszewska).

1567-5394/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2003.09.024

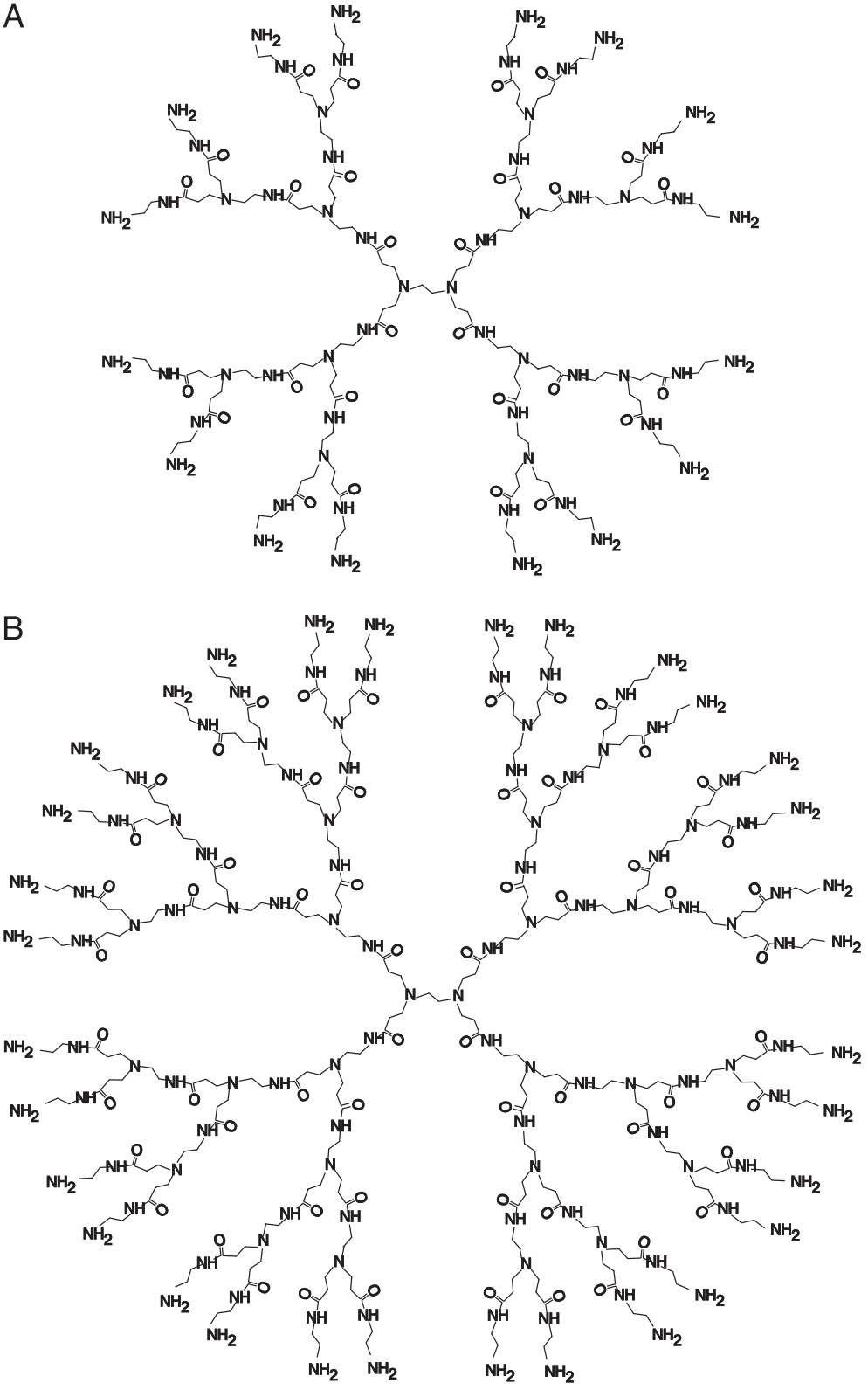

Fig. 1. The PAMAM dendrimer structures: (A) PAMAM G2; (B) PAMAM G3; (C) PAMAM G4.

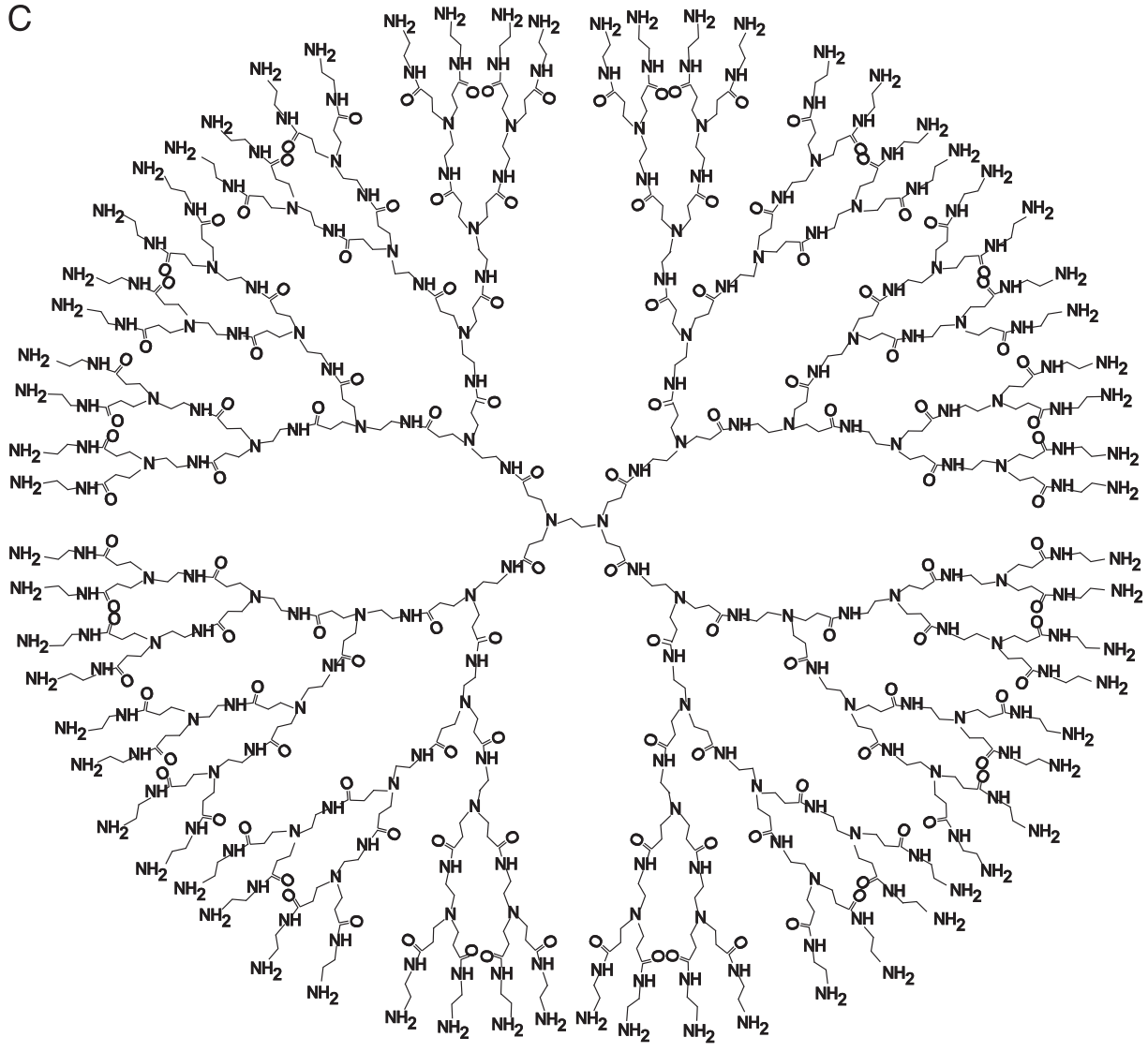

Fig. 1. (*continued*).

Changes in fluorescence intensity and steady-state fluorescence anisotropy of TMA-DPH and 12-AS were monitored. The anisotropy was calculated according to the equation:

$$r = \frac{I_{vv} - I_{vh}G}{I_{vv} + 2I_{vh}G},$$

where I_{vv} and I_{vh} are the components of emitted light intensity, respectively, parallel and perpendicular with reference to the direction of polarization of the excitation light and G is the correction factor used to correct for unequal transmission in the optical system:

$$G = \frac{I_{hv}}{I_{hh}}.$$

3. Results and discussion

Both TMA-DPH and 12-AS are fluorescent probes commonly used to evaluate membrane fluidity due to their incorporation into lipid bilayer. The easiest parameter to measure, connected with fluorophore motions, is steady-state anisotropy of fluorescence [4]. Fluorescence anisotropy is inversely related to membrane fluidity.

In our work, TMA-DPH and 12-AS were used to study changes in their rotational freedom in the presence

Table 1
Characterisation of used dendrimers

Name, generation	Terminal groups	Number of terminal groups	Molecular weight (Da)	Diameter (nm)
PAMAM G2	$-NH_2$	16	3256	2.7
PAMAM G3	$-NH_2$	32	6909	3.6
PAMAM G4	$-NH_2$	64	14,125	4.5

of dendrimers. Generally, for both probes the higher dendrimer concentrations were used the higher values of anisotropy were obtained (Fig. 2). However, the changes were much more significant for 12-AS. Anisotropy enhancement was observed even for the lowest dendrimer concentrations when the number of 12-AS moles per 1 mol of dendrimer equalled to 100 and 10. Changes in anisotropy were accompanied by a rapid increase of fluorescence intensity (Fig. 3). This suggests that 12-AS molecules incorporated into dendrimer cavities and were located in more rigid environment which caused a restriction of rotational freedom. The similar incorporating effect had earlier been observed for 1-anilinonaphthalene-8-sulfonic acid (ANS) [5].

It is believed that higher generations of dendrimers are more capable of encapsulating guest molecules because their structure is denser [6]. It is interesting that such a dependence was not observed in our experiments. In most cases, the size of the dendrimer had no impact on its host ability.

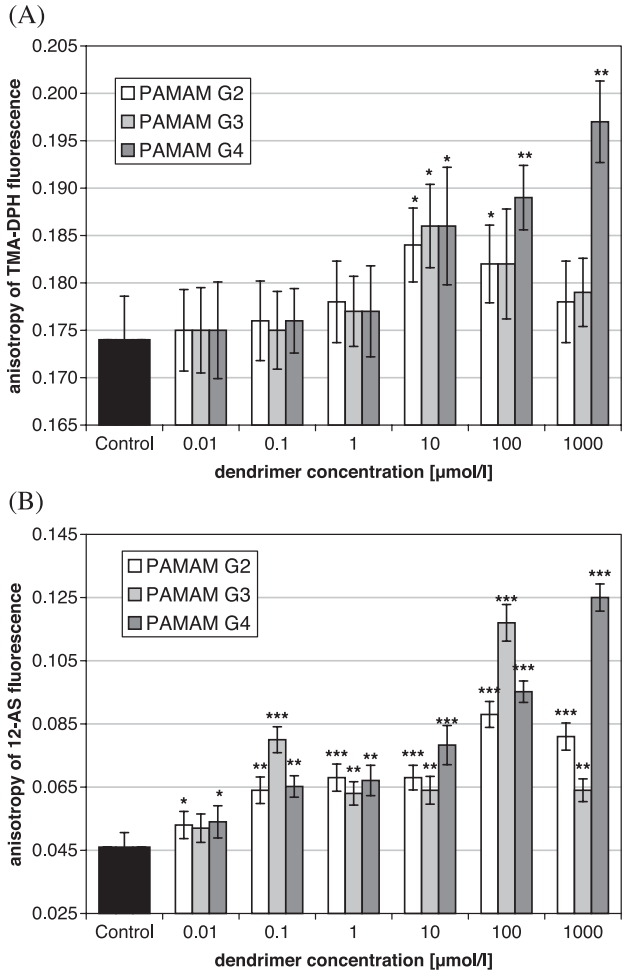

Fig. 2. The effect of PAMAM dendrimers on anisotropy of TMA-DPH (A) and 12-AS (B). Results are expressed as means ± S.D. of five experiments. Statistical significance was assessed using Student–Fisher test, $*P<0.05$, $**P<0.01$, $***P<0.001$.

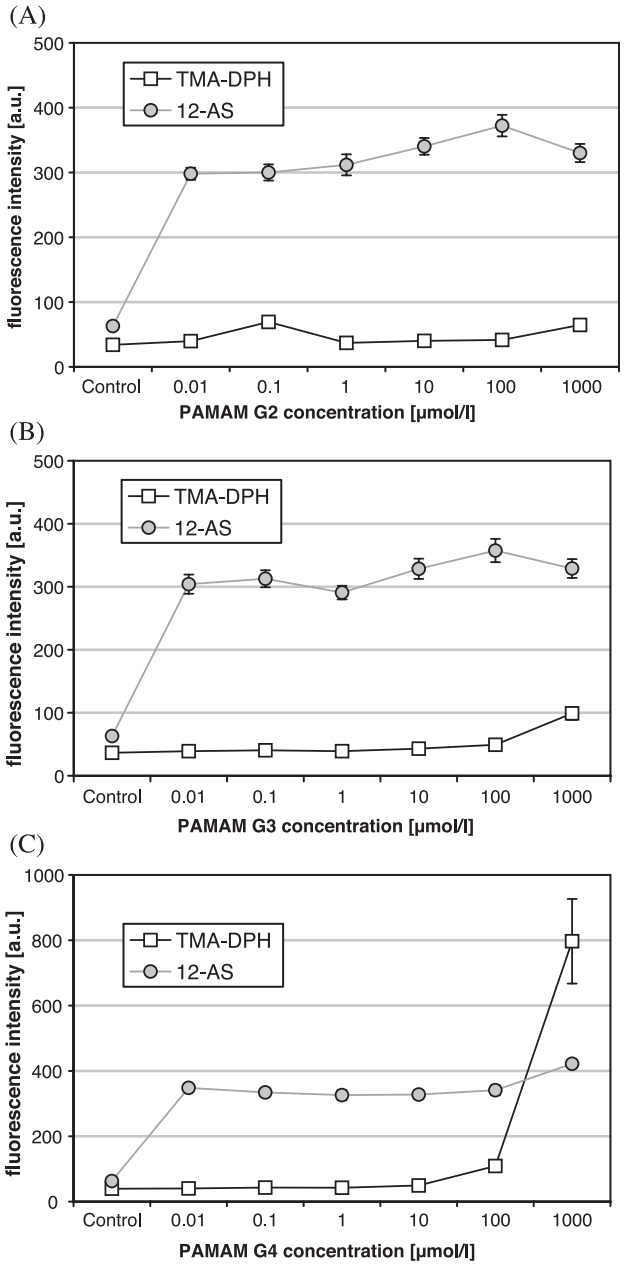

Fig. 3. Changes in fluorescence intensity of TMA-DPH and 12-AS upon addition of dendrimers: (A) PAMAM G2; (B) PAMAM G3; (C) PAMAM G4. Results are expressed as means ± S.D. of five experiments.

Significant changes in TMA-DPH anisotropy were observed only for the highest dendrimer concentrations (10, 100 and 1000 µmol/l) when more dendrimer molecules than dyes molecules were in a system. It indicates that TMA-DPH was not encapsulated. Observed changes may be connected with interdendrimer interactions in solution. It was found that amino-terminated dendrimers did not behave as a single homogenous species. On contrary, they form oligomeric aggregates [7]. As these polymers have a large positive charge on their surfaces, the nature of the self-association remains unclear. It is possible that TMA-DPH molecules aggregated on the surface of dendrimer enhancing the self-

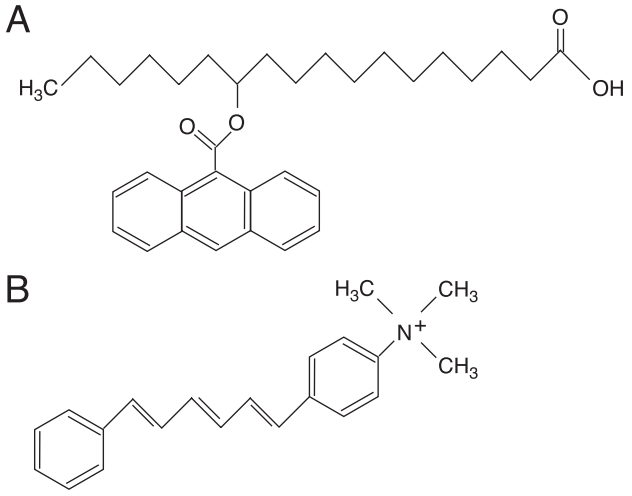

Fig. 4. The structures of 12-AS (A) and TMA-DPH (B).

association process. Forming large aggregates could cause a restriction of rotational freedom. This process was especially considerable for the highest concentrations of PAMAM G4 where the tremendous increase of fluorescence intensity was also recorded.

Generally, more profound changes in fluorescence intensity and anisotropy were observed for 12-AS. We believe that this probe has better properties for being encapsulated into PAMAM dendrimers because it possesses a carboxylic group. Electrostatic attractions between a positively charged dendrimer surface and a negatively ionised carboxylic group of 12-AS may help in the first stage of incorporation.

4. Conclusions

Checking the guest–host properties of two fluorescent probes in PAMAM dendrimers, we found the different behaviour for 12-AS and TMA-DPH. It may be due to differences in dye structures. 12-AS is a non-polar fatty acid derivative whereas TMA-DPH is an amphiphilic salt possessing positive charge (Fig. 4). Electrostatic forces can be responsible for protecting TMA-DPH from incorporation into dendrimers.

References

[1] D.A. Tomalia, H. Baker, J.R. Dewald, M. Hall, G. Kallos, S. Martin, J. Ryder, J. Ryder, P. Smith, A new class of polymers: Starburst-dendritic macromolecules, Polym. J. 17 (1985) 117–132.
[2] J.F.G.A. Jansen, E.M.M. de Braber van den Berg, E.W. Meijer, Encapsulation of guest molecules into a dendritic box, Science 266 (1994) 1226–1229.
[3] C. Kojima, K. Kono, K. Maruyama, T. Takagishi, Synthesis of polyamidoamine dendrimers having poly(ethylene glycol) grafts and their ability to encapsulate anticancer drugs, Bioconjugate Chem. 11 (2000) 910–917.
[4] C.D. Stubbs, B.W. Williams, Fluorescence in membranes, in: J.R. Lakowicz (Ed.), Topics in Fluorescence Spectroscopy, vol. 3. Plenum, New York, 1992, pp. 231–272.
[5] B. Klajnert, M. Bryszewska, The interaction of tryptophan and ANS with PAMAM dendrimers, Cell. Mol. Biol. Lett. 7 (2002) 1087–1094.
[6] A.M. Naylor, W.A. Goddard III, Starburst dendrimers: 5. Molecular shape control, J. Am. Chem. Soc. 111 (1989) 2339–2341.
[7] A. Nourse, D.B. Millar, A.P. Minton, Physicochemical characterization of generation 5 polyamidoamine dendrimers, Biopolymers 53 (2000) 316–328.