Single-Molecule Study of Dendrimer-Hydrocarbon Interaction

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ABSTRACT

In this paper we report our study on the interaction of dendrimer and hydrocarbon. The absorbance of generation-3 dendrimer PAMAM (0.25%) was reduced by ~5% in 60 min with the addition of hydrocarbon Squalane (0.405 mg/mL), possibly due to the phase separation between the PAMAM remained in the solution and the PAMAM bound to the Squalane. Using single-molecule fluorescence microscopy we found that PAMAM molecules in the solution could be immobilized by Squalane molecules coated on a glass substrate, as indicated by the much increased fluorescence intensity of the TRITC-labeled PAMAM in an evanescent wave field. Our study offers the first evidence at the single-molecule level on dendrimer and hydrocarbon interaction and facilitates our understanding of using dendrimers for the prevention of gas hydrates for the petroleum industry.

Keywords: PAMAM, Squalane, fluorescence

1. INTRODUCTION

Dendrimers are a class of monodisperse, branched, and radially symmetric macromolecules [1-3]. The branches of dendrimers, organized into layers or "generations", stem out from an interior core to an exterior surface of multiple functional groups. The terminal groups of dendrimers can be modified to elicit desirable chemical and biological functionalities. When constructed into sufficient size, dendrimers may contain cavities within their structure which allow for the encapsulation of compounds and drug molecules.

Dendrimers as branched nonlinear molecules have found many applications in polymer science and the polymer industry. Within the exciting field of biomedical research, dendrimers have been utilized as diagnostic and therapeutic tools, and fill in such roles as drug delivery systems, imaging probes, and carriers of genetic material. In addition, dendrimers have shown promise in the areas of catalyst chemistry, superconducting materials, and chemical sensing [4-6].

Recently it has been discovered that dendrimers and their closely related hyperbranched polymers can effectively prevent the formation of ice crystals in oil pipelines, a major issue which costs million dollars a day for the petroleum industry. However the underlining mechanism of this application is still poorly understood, especially at the single-molecule level. Here we present our absorption spectrophotometry and total-internal-reflection-fluorescence (TIRF) microscopy studies of the interaction of cationic generation-three polyamidoamine (G3-PAMAM) dendrimers and hydrocarbon Squalane. Using spectrophotometry we show that the mixing of Squalane with G3-PAMAM induced the enhancement of PAMAM absorption peak at 506 nm, suggesting that the hydrocarbon was adsorbed by PAMAM through hydrophobic-hydrophobic interactions. Using TIRF we found that TRITC-labeled G3-PAMAM molecules could be separate from the liquid phase and immobilized onto a glass substrate pre-coated with Squalane. These studies were validated by our transmission electron microscopy (TEM) study which showed the formation of PAMAM-Squalane complexes. Our studies suggest that the morphology of dendrimers significantly promotes the adsorption of hydrocarbon through hydrophobic-hydrophobic interactions, thus shielding them from exposure to water that induces gas hydrates.

2. RESULTS AND DISCUSSION

We used three different techniques, absorbance spectroscopy, TEM, and TIRF microscopy, to probe the interaction between PAMAM and Squalane. A PAMAM molecule is constructed from an ethylenediamine-core and possesses amine terminated branches. Noticeable cavities occur within the dendrimer when the molecule is constructed beyond generation 3. The chemical

structure of a G3-PAMAM is shown in Figure 1a. The chemical structure of Squalane, a linear hydrocarbon chain, is shown in Figure 1b. G3-PAMAM (in water, MW 6,909) was obtained from Dendritech as a model dendrimer and Squalane (Aliphatic hydrocarbon kit 18, MW 422 g/mol) was purchased from Sigma as a model hydrocarbon for our study. The surface of the G3-PAMAM was modified by replacing the amino group by 5(6)-TRITC (Anaspec) which absorbs and fluoresces at 543 and 571 nm respectively. G3-PAMAM was mixed with TRITC at a 1:2 molar ratio and the mixture was then incubated at room temperature overnight. The labeled G3-PAMAM was purified using membrane filters (Millipore, Microcon YM-3) to remove free TRITC.
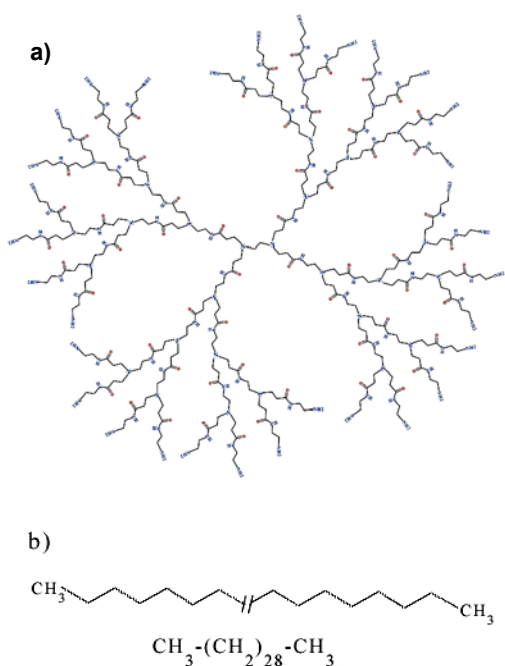

Figure 1. Structures of a) G3-PAMAM and b) Squalane.

2.1 Absorbance Spectroscopy

Various generations of PAMAM absorb strongly at the lower wavelength range of the UV spectrum as measured using a spectrophotometer (Biomate 3) (Figure 2a), while TRITC-labeled G3-PAMAM absorbs at ~506 nm (Figure 2b). This is probably due to the presence of TRITC which absorbs at 543 nm at the surface of the G3-PAMAM. In other words the shift of absorbance can be attributed to the formation of dendrimer-TRITC complexes.
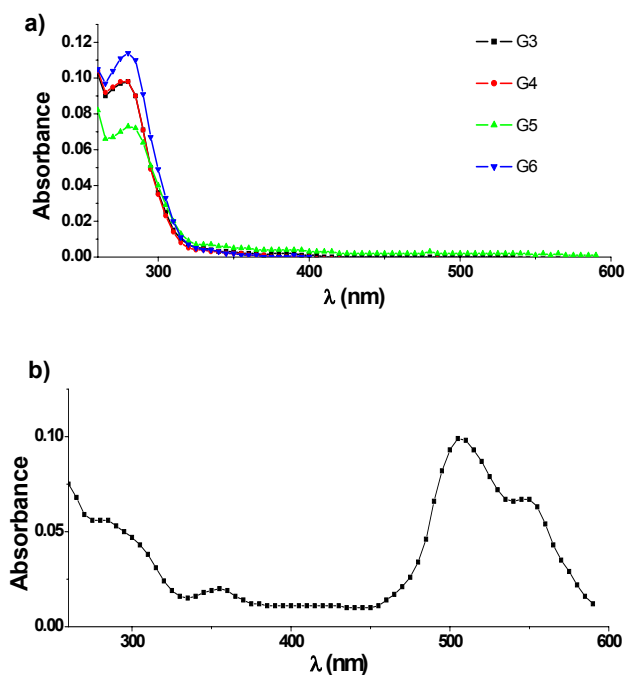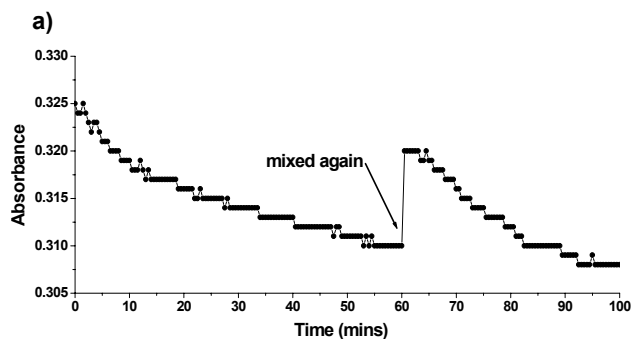

Figure 2. Absorption spectra of a) various generations of PAMAM at 0.25 w% in water. PAMAM primarily absorb at ~280-285 nm; b) TRITC-labeled G3-PAMAM (0.25 w% in water).

The interaction between labeled G3-PAMAM and Squalane (0.405 mg/mL) was measured at 506 nm. The time dependence of TRITC-PAMAM absorbance due to the presence of Squalane is shown in Figure 3a. The scheme of induced PAMAM phase separation is proposed in Figure 3b.
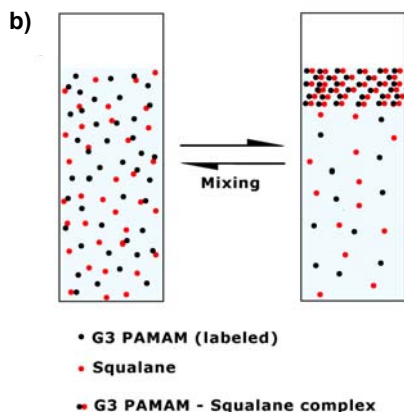

2

b)

- G3 PAMAM (labeled)
- Squalane
- G3 PAMAM - Squalane complex

Figure 3. a) Time dependence of G3-PAMAM-Squalane interaction measured at λ=506 nm for 0.75% w/w G3-PAMAM in water. b) The decrease in the absorbance of G3-PAMAM was probably due to the low density of Squalane which dragged the PAMAM along to the surface of the solution.

2.2 TEM Imaging

TEM images were acquired for samples containing G3-PAMAM and G3-PAMAM along with Squalane as shown in Figure 4. The much enlarged sizes of the nanoparticles in Figure 4b, as compared to those in Figure 4a, supports the hypothesis that strong interactions occurred between dendrimers and the hydrocarbon Squalane.
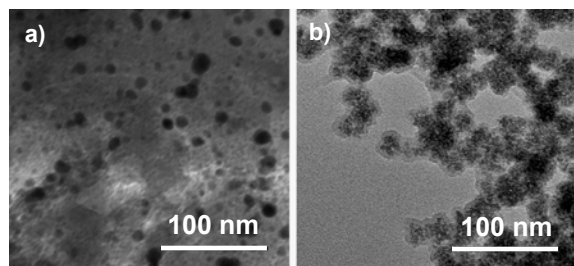

Figure 4. TEM graphs of a) 0.25% w/w of TRITC-labeled G3-PAMAM and b) 0.25% w/w TRITC-labeled G3-PAMAM. The concentration of the Squalane was diluted by 2000 times from the stock of 0.806 mg/mL.

2.3 TIRF Imaging

The binding between PAMAM and Squalane was imaged using a TIRF microscope. A glass substrate was pre-cleaned with 2% Micro-90 detergent and Millipore water. Then the surface was coated with Squalane (10,000 times dilution of the stock solution) using a spin coater running for 1 min

at 2000 rpm. TRITC-labeled G3-PAMAM at 26 nM was let react with this surface for 15 min and then washed with Millipore water. TRITC-labeled G3-PAMAM would fluoresce when bound to or within 100 nm from the glass substrate, which is the range of the evanescent field excited by a Nd:YAG laser at 532 nm.
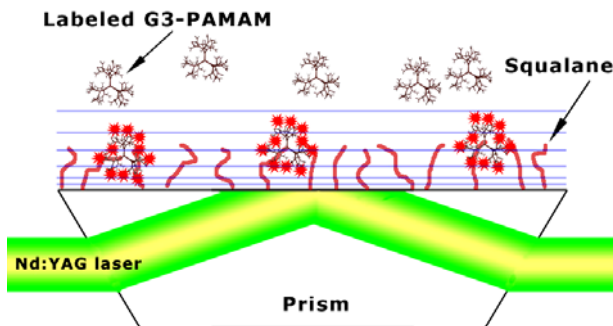

Figure 5. Scheme of exciting TRITC-labeled G3-PAMAM for probing the interaction of PAMAM and Squalane. The glass substrate was coated with Squalane. The TIR condition was ensured by controlling the laser beam beyond the critical incidence angle.
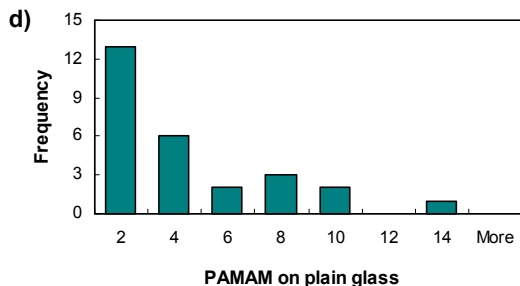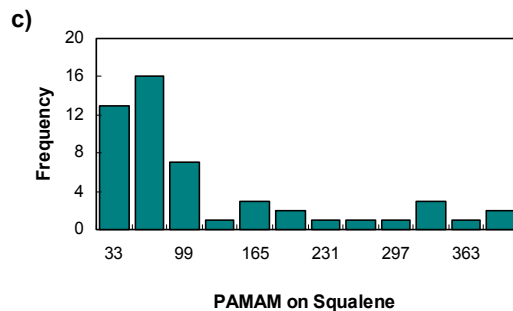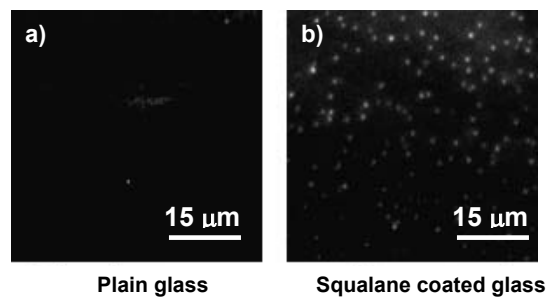

Plain glass　　　　**Squalane coated glass**

3

Figure 6. Comparison of TRITC-labeled G3-PAMAM a) without and b) with the presence of Squalane. The plain glass in a) shows few G3-PAMAM molecules bound while the Squalane-coated glass in b) shows much enhanced binding of TRITC-labeled G3-PAMAM. c) Histograms of TRITC-labeled G3-PAMAM population on Squalane-coated glass surface and d) plain glass surface.

3. CONCLUSIONS

The interaction between dendrimers and hydrocarbons are hypothesized to be due to the entrapment of hydrocarbons by the branches of dendrimers through hydrophobic-hydrophobic interactions. We have shown that Squalane interacts with PAMAM by the combined methods of absorbance, TEM, and single-molecule imaging. This study is a first step towards unraveling the underlining mechanism of this interaction. We will conduct molecular simulations to complement our future experimental studies on the interaction kinetics of PAMAM and Squalane, which may have implications for advancing polymer science as well as for the petroleum industry.

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4