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Detecting the Adsorption of Dye Molecules in Homogeneous Poly(propylene imine) Dendrimer Monolayers by Surface Plasmon Resonance Sensor

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Received September 6, 2001

Abstract: In this work, we studied guest−host interactions between various dye molecules and the fifth-generation poly(propylene imine) (PPI-5) dendrimers in aqueous solutions using a surface plasmon resonance (SPR) sensor. The effect of the properties of guest and host molecules (e.g., charge and shape) and media (e.g., pH and ion strength) on affinity between guest and host molecules was investigated. Based on an immobilized homogeneous monolayer of PPI-5 dendrimer tethered to carboxyl-terminal self-assembled monolayers, the adsorption behavior of a group of dye molecules in PPI-5 was obtained. Results show that the strong affinity of PPI-5 to Rose Bengal and erythrosine B is attributed to the good match in charge and shape between the cavities of the dendrimer and the dye molecules. Maximum adsorption around a pH value of 7 was observed. The kinetic behaviors of different dye molecules in dendrimers were also studied. A fundamental understanding of guest−host interactions in dendrimers will guide the design of new-generation sensors and drug delivery carriers.

Introduction

Dendrimers have been regarded as interesting candidates for applications in guest−host chemistry because of their unique cavities.[1−4] The size, shape, and chemical microenvironment of these cavities can be controlled by synthetic procedures developed for dendrimer preparation.[5,6] The properties of guest and host molecules, as well as of the media (e.g., solvent) that guest and host molecules are in, affect the guest−host interactions dramatically.

Both experimental and molecular simulation studies have shown that 2,4-dichlorophenoxyacetic acid and acetylsalicylic acid can be adsorbed into polyamidoamine (PAMAM) dendrimers when the solvent is $CDCl_3$.[7] Meijer and co-workers have demonstrated that poly(propylene imine) (PPI) dendrimers can adsorb different dye molecules from CH_2Cl_2 solvent and further encapsulate them inside the so-called dendrimer box, which is the PPI dendrimers capped with *tert*-butyloxycarbonyl-L-Phe (tBOC-L-Phe).[8,9] In these two experiments, there exists a difference in polarity and/or hydrophobicity between internal (cavity) and external (solvent) environments of dendrimers and solution concentration is high. Thus, these experimental results mainly show the maximum capacity of cavities to adsorb guest molecules but are not sensitive to guest molecules at different charge states. For example, each fifth-generation PPI dendrimer box can encapsulate up to four anionic dye molecules, such as Rose Bengal, or cationic dye molecules, such as methylene violet 3RAX and rhodamide B.[8,9] In contrast, it is found that nonpolar pyrene molecules prefer to stay inside the cavities of a low-polarity dendrimer rather than in external water solvent of high polarity.[10] All these experiments suggest that the charge state and polarity of guest and host molecules and solvent affect the guest−host interactions.

The shape of guest molecules and dendrimer cavities also plays an important role in the guest−host interactions. The dendrimer box can encapsulate up to four Rose Bengal, methylene violet 3RAX, or rhodamide B dye molecules, which have a triangular shape, while it can encapsulate only up to 2.6 new coccine dye molecules, which have a dumbbell shape.[8,9] The fifth-generation PPI dendrimers modified with nonpolar palmitoyl chains show high selectivity in extracting Rose Bengal over fluorescein dye molecules from aqueous phase to organic phase,[11] which may be caused by a better topologic match between Rose Bengal molecules and the cavities of dendrimers.

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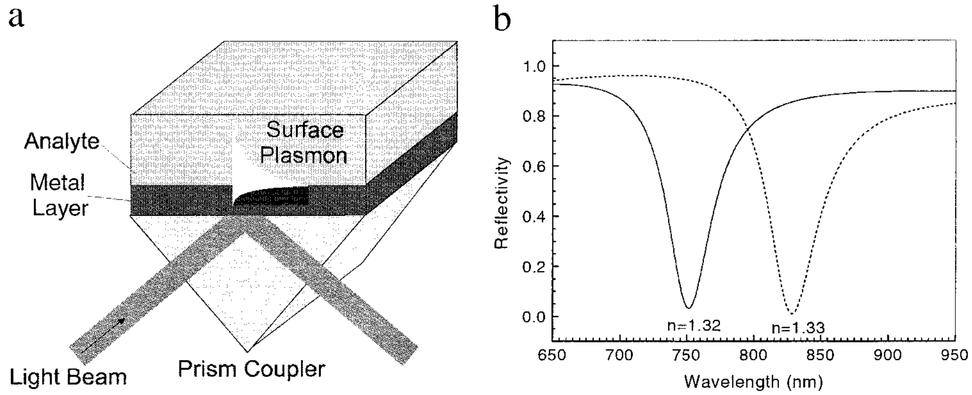

Figure 1. (a) Prism-based SPR sensor with wavelength interrogation consisting of a prism coupler and a thin SPR-active metal layer. (b) Typical spectra calculated for two different refractive indices before and after the introduction of an analyte for the SPR sensor consisting of an SF14 glass prism and a thin gold layer.

When small guest molecules are used, for example, various volatile organic compounds (VOCs) in PAMAM,[12] it is difficult to determine the relationship between the unique shapes of the major cavities of PAMAM and of guest molecules.

Besides equilibrium thermodynamic properties, the kinetic effect should also be taken into account in studying guest−host interactions since most applications of dendrimers, such as in drug delivery, sensing, separation, and catalysis, involve dynamic processes. For example, dendrizymes were designed to have a catalytic center surrounded by dendrimer branches to create different internal and external environments to promote catalytic activities.[13] Many experimental results showed that the activity of the catalytic center decreased with the increase of dendrimer generations.[1] We speculate that this is due to low diffusion rates of reactants and products through dendrimers.

Optical sensors based on surface plasmon resonance (SPR) are becoming increasingly popular since they allow for real-time monitoring of processes occurring at solid surfaces.[14−16] SPR sensors have been extensively used for study of biomolecular interactions between free analyte molecules in a solution and their counterparts immobilized on the sensor surface. With the same instrument used in this work, 5 and 0.5 ng/mL amounts of staphylococcal enterotoxin B (SEB) can be detected directly through SEB and through secondary antibody amplification, respectively.[17] To the best of our knowledge, this is the first time that an SPR sensor has been used to study the guest−host interactions in dendrimers.

In this work, guest−host interactions between fifth-generation poly(propylene imine) (PPI-5) dendrimers and various dye molecules in aqueous solutions were studied using an SPR sensor. PPI-5 dendrimer monolayers were immobilized on the SPR sensor surface via carboxyl-terminal self-assembled monolayers (SAMs). The uniform monolayers were characterized by atomic force microscopy. Seven dye molecules were chosen to study the effects of the charge states and molecular shapes of guest molecules on guest−host interactions in dendrimers. Water was used as a solvent to provide strongly polar media. The effect of solution pH value on guest−host interactions and kinetic behaviors of different dye molecules in dendrimers were also investigated.

Methods and Materials

SPR Sensor. A high-resolution SPR instrument developed at the University of Washington was used in this work. Unlike most SPR sensors that are based on angular measurements,[18,19] our SPR sensor utilizes wavelength interrogation.[20,21] In this configuration, a polychromatic light beam passes through an optical prism with a thin metal layer and excites surface plasmons at the interface between the metal layer and the analyte (Figure 1a).

The excitation of the surface plasmon is accompanied by the transfer of optical energy into surface plasmons and their dissipation in the metal layer, resulting in a narrow dip in the spectrum of reflected light (Figure 1b). The wavelength at which the resonant excitation of the surface plasmons occurs depends on the refractive index of the analyte in the proximity to the SPR surface. In this work, PPI-5 dendrimers were immobilized on the SPR sensor surface. Interactions between the target analytes (dye molecules) and PPI-5 dendrimers give rise to a local refractive index change. The amount of captured analytes in dendrimers can be quantified by measuring the refractive index change-induced shift in the resonant wavelength. Figure 2 presents the theoretical dependence of the resonant wavelength shift on the occupancy of PPI-5 cavities with Rose Bengal assuming 20% porosity of the dendrimer layer. Figure 2 indicates that a resonant wavelength shift of about 10 nm is generated when each PPI-5 dendrimer captures four Rose Bengal molecules (100% occupancy) in its cavities.

In our SPR instrument (Figure 3), the resonant wavelength was determined by means of a high-resolution optical spectrograph (Ocean Optics). A generic SPR chip was prepared by coating a glass substrate with an adhesion-promoting chromium layer (thickness ~2 nm) and a surface-plasmon-active gold layer (thickness ~50 nm) using electron beam evaporation in a vacuum. This procedure was followed by further surface functionallization. The chip was attached to the base of the prism, and optical contact was established using a refractive index matching fluid (Cargille). A dual-channel Teflon flow cell containing two independent parallel flow channels with small chambers was used to contain a liquid sample during the experiments. A peristaltic pump (Ismatec) was utilized to deliver a liquid sample to the two chambers of the flow cell. The dilute dye solutions of 2 μg/mL in phosphate-buffered saline (PBS) solution at pH 7.4 were flowed over PPI-5 dendrimer monolayer surfaces at a flow rate of 0.05 mL/min.

Tapping-Mode Atomic Force Microscopy (TM-AFM). All TM-AFM images were acquired using Digital Instruments (DI) Multimode

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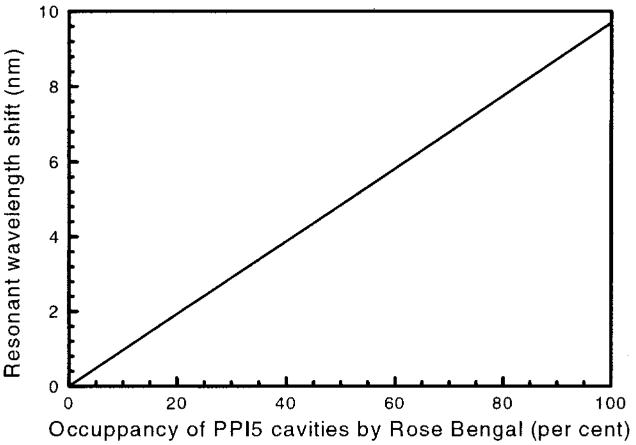

Figure 2. Simulated response of an SPR sensor with an immbolized PPI-5 dendrimer monolayer on the sensor surface to the adsorption of Rose Bengal. The resonant wavelength shift as a function of the percentage of PPI-5 cavities occupied by Rose Bengal was calculated for an SPR sensor consisting of an SF14 glass prism, a gold layer (thickness of 50 nm), a PPI-5 monolayer (refractive index of 1.518, thickness of 4 nm, and porosity of 20%), Rose Bengal (refractive index of 1.73), and water. Sensor operating wavelength is 750 nm.

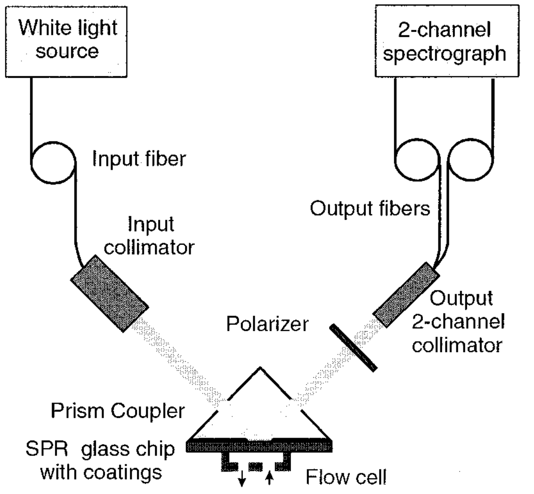

Figure 3. Experimental setup for a dual-channel SPR sensor.

Nanoscope IIIa (Santa Barbara, CA) equipped with a 10 μm “E” scanner. Commercial Si cantilevers (TESP, DI) with resonant frequencies of ~270 kHz, force constants of 20−100 N/m, and tip apex radii of 5−10 nm were used. Images were recorded with typical scan rates of 1.0−2.0 Hz. All experiments were performed in air under a relative humidity less than 40%.

Chemical Modification of SPR Chips. A monolayer of the PPI-5 dendrimer was prepared by tethering the dendrimer to carboxyl-terminated SAMs on a gold-coated glass substrate. To vary the density of PPI-5 dendrimers on SAM-modified surfaces, long-chain carboxyl-terminated thiol solutions were mixed with short-chain hydroxyl-terminated thiol solutions with molar compositions of 1%, 10%, 30%, and 100%. Specifically, a gold-coated glass substrate was cleaned by washing with chloroform and ethanol, cleaning under UV light, washing with water and ethanol, and drying. Mixed SAMs were formed by soaking a cleaned substrate in a total 1 mM ethanolic solution of carboxyl- and hydroxyl-terminated thiols for 24 h, rinsing extensively with ethanol, and drying in a stream of N_2. Carboxyl-terminal groups on a SAM-modified substrate were activated by soaking the substrate in a mixed solution of 2 mg/mL *N*-hydroxysuccinimide (NHS) and 2 mg/mL 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for 1 h. The NHS-activated substrate was then incubated with a PPI-5 aqueous solution (~0.2 mg/mL) for 24 h. The dendrimer-functionalized substrate was washed by deionized water extensively and dried under a mild stream of N_2.

Chemicals. Amine-terminated PPI-5 dendrimer was purchased from Sigma-Aldrich as a 10 wt % solution in methanol. The solution was stored at 4 °C and used as received. Rose Bengal (**I**), erythrosin B (**II**), 4,5,6,7-tetrachlorofluorescein (**III**), fluorescein (**IV**), methlene violet 3RAX (**V**), rhodamide B (**VI**), new coccine (**VII**), 16-mercaptohexadecanoic acid ($HSCH_2(CH_2)_{14}COOH$), 11-mercapto-1-undecanol ($HSCH_2(CH_2)_{10}OH$), 2-aminoethanethiol hydrochloride ($HS(CH_2)_2NH_2$), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and 10 mM PBS (138 mM NaCl, 2.7 mM KCl at pH 7.4) were all purchased from Sigma-Aldrich and used as received.

Results and Discussion

Immobilized Dendrimer Monolayers. To detect the adsorption of dye molecules in the PPI-5 dendrimer using an SPR sensor, a homogeneous dendrimer monolayer needs to be immobilized on the sensor surface. Wells and Crooks reported methods for the covalent attachment of PAMAM dendrimers to SAMs of mercaptoundecanoic acid on gold via forming amide bond[22] and to mixed SAMs consisting of a low surface concentration of longer-chain *n*-alkanethiol terminated with a reactive group, such as mercaptoundecanoic acid diluted by a shorter, unreacted methyl-terminated thiol.[12,23,24] Activation of the small percentage of acid groups results in linking one or just a few terminal groups of each dendrimer to the SAMs. This significantly reduces dendrimer deformation. Fréchet and coworkers reported the attachment of modified poly(benzyl ether) dendrimer monolayers to a silicon substrate via covalent or ionic bonding.[25−27]

We followed the methods used by Crooks et al.[22−24] to covalently attach amino-terminal PPI-5 dendrimers to carboxyl-terminal SAMs via forming amide bonds to form dendrimer monolayers. To form amide bonds instead of simply ionic bonds due to an acid/base self-assembly process,[27,28] carboxyl groups on SAMs were activated using NHS. Formation of covalent bonds between carboxyl and amino groups was verified using FTIR.[24] The surface density of PPI-5 was determined by the availability of carboxyl-terminal groups on mixed SAMs, which is proportional to the composition of carboxyl-terminated thiol in the solution containing thiol mixtures, although the preferential adsorption of long-chain thiols on the surface over in the solution was observed.[29] It is clearly shown in TM-AFM images (Figure 4) that the covalently bonded PPI-5 dendrimers on SAMs-modified surfaces increase as more carboxyl groups are available on the surfaces. When the mixed SAM was prepared by 1% composition of $HSCH_2(CH_2)_{14}COOH$ mixed with $HSCH_2(CH_2)_{10}OH$ in solution, the surface composition of $HSCH_2(CH_2)_{14}COOH$ was very low. Thus, individual PPI-5 dendrimers linked to the carboxyl groups on the mixed SAM surface are easily identified, as shown in Figure 4a. When the

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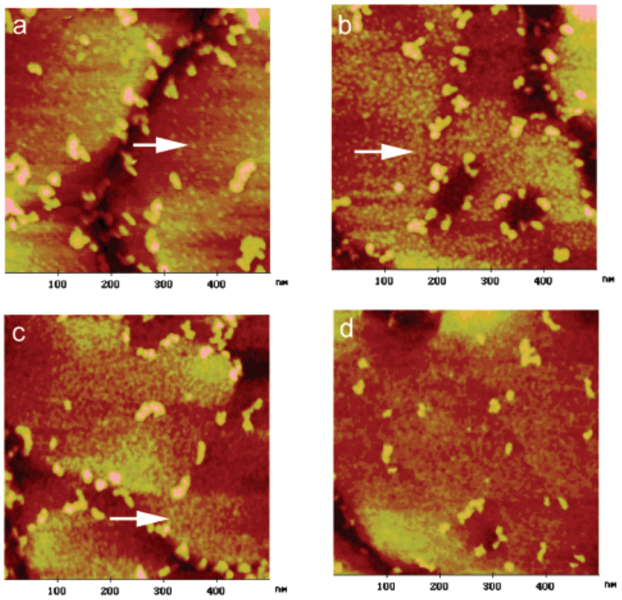

Figure 4. Tapping-mode AFM images of PPI-5 dendrimers covalently bonded on mixed SAMs of $HSCH_2(CH_2)_{14}COOH$ and $HSCH_2(CH_2)_{10}OH$ on Au(111) with a solution molar composition of 1% (a), 10% (b), 30% (c), and 100% $HSCH_2(CH_2)_{14}COOH$ (d).

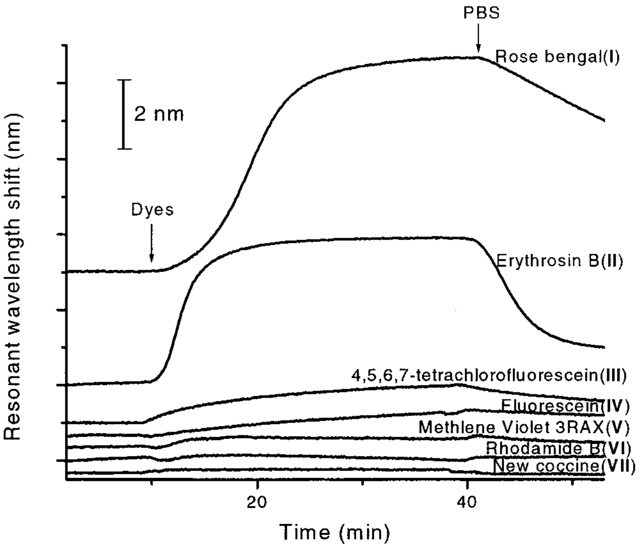

Figure 5. Responses of an SPR sensor with PPI-5 dendrimer monolayer-modified surfaces to different dye molecules in PBS buffer (pH 7.4) solutions. The sensor was first stabilized by a PBS buffer (pH 7.4) solution for 10 min and then flowed by a dilute dye solution for 30 min, and finally washed by a PBS (pH 7.4) solution.

solution composition of $HSCH_2(CH_2)_{14}COOH$ increased to 10% and 30%, more carboxyl groups were available on the surfaces, and the surface density of PPI-5 dendrimers increased. Individual PPI-5 dendrimers still were identified in the TM-AFM images (Figure 4b,c), although the diameter of dendrimers appeared to be slightly smaller as surface coverage increased due to the effect of AFM tip convolution.[30] When the SAM surface was 100% carboxyl groups, PPI-5 dendrimers covered almost all of the surface, and the surface was very flat, as seen in Figure 4d, indicating that a uniform PPI-5 dendrimer monolayer was formed.

To form a uniform PPI-5 dendrimer monolayer, the step of activating carboxyl groups and the solvent used in cross-linking PPI-5 dendrimers to carboxyl-terminated surface are very important. Very large aggregated PPI-5 particles were observed in our experiments if PPI-5 dendrimers were directly adsorbed on carboxyl-terminated SAM without being further activated. Different solvents were used in our experiments in cross-linking PPI-5 dendrimers to carboxyl-terminated surface. Very rough dendrimer-modified surfaces were observed in TM-AFM images if ethanol was used as a solvent, which may be caused by forming multilayers of dendrimers. In contrast, a very smooth surface was obtained if water was used, which indicates that a monolayer of dendrimers was formed. We chose water as a solvent to cross-link PPI-5 to the SAM surface, although water molecules could partially hydrolyze NHS ester and decrease the reaction efficiency between PPI-5 and NHS ester.[31]

The homogeneous covalently bonded PPI-5 dendrimer monolayers on 100% carboxyl-terminated SAM-modified Au-coated substrates were used in our studies of the adsorption of all dye molecules in PPI-5 dendrimers. PPI-5 dendrimer monolayers tethered on SAMs is very stable. In our SPR experiments, when the flow rate, pH value, and ion strength were changed from 0.05 to 0.2 mL/min, 1 to 13, and 0.138 to 1 M NaCl, respectively, no resonant wavelength shift was observed, indicating the stability of the dendrimer monolayers.

Affinity of Dyes to PPI-5 Dendrimers. Seven dyes (**I**−**VII**) were chosen as guest molecules to study the guest−host interactions with the PPI-5 dendrimer. A dilute dye solution was flowed over the PPI-5 dendrimer-modified SPR sensor surface. The effects of the charge and shape of both dye molecules and dendrimers, solution pH value, and kinetics on dye−dendrimer interactions were investigated. The SPR responses for all dyes adsorbed in PPI-5 dendrimers are shown in Figure 5. The relative SPR responses for dyes **I**−**VII** are 1, 0.67, 0.26, 0.11, 0.05, 0.03, and 0.02, respectively. Both Rose Bengal (**I**) and erythrosin B (**II**) had significantly high affinity to PPI-5 dendrimers. The affinity of 4,5,6,7-tetrachlorofluorescein (**III**) and fluorescein (**IV**) to PPI-5 dendrimers is lower than those of dyes **I** and **II** but almost one order of magnitude higher than those of other three dyes (**V**−**VII**). In our experiments, 100 times concentrated Rose Bengal solutions were used to detect the saturated response (the plateau of each SPR response vs time curve). No obvious difference was found, indicating that the saturated responses shown in Figure 5 are the maximum adsorption of dyes.

The molecular structures of these seven dyes are given in Figure 6a. Of seven dye molecules, five (**I**−**IV** and **VII**) are anionic and two (**V** and **VI**) are cationic. When a PBS solution (pH 7.4) was used as a carrier fluid, all amino groups at the outer surface and some interior tertiary amines of PPI-5 dendrimers are partly protonated.[32] Thus, all anionic dyes, except for **VII**, have higher affinity to PPI-5 than cationic dyes. This charge-determined affinity to PPI-5 dendrimer was observed in extraction experiments when PPI dendrimers modified with apolar end groups were used as extractant. Cationic dye, such

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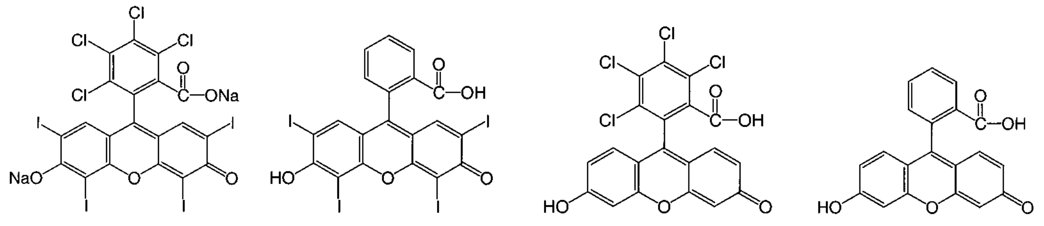
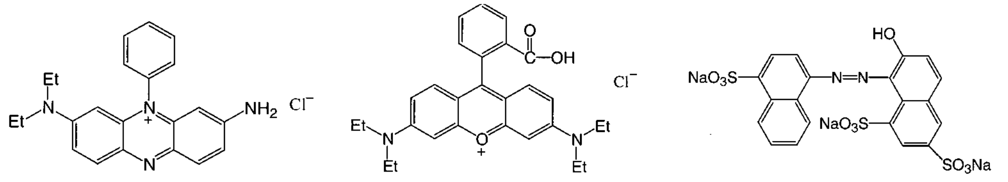


Rose Bengal (**I**) Erythrosin B (**II**) 4,5,6,7- Tetrachlorofluorescein (**III**) Fluorescein (**IV**)



Methylene violet 3RAX (**V**) Rhodamide B (**VI**) New coccine (**VII**)

Substructure **i** Substructure **ii**

Substructure **iii** Substructure **iv**

Figure 6. Kekule structures of various dye molecules used in this work (a) and some of their substructures (b).

as ethidium bromide, cannot be extracted at all from an aqueous phase into an organic phase, while anionic dyes, such as **I**−**IV**, can.[11] This indicates that the matching of charges between host and guest plays an important role in guest−host interactions. However, charge is not the only factor. Topologic matching between dendrimer cavities and dye molecule shapes is also very important. The affinity of the most negatively charged **VII** is about 50 times lower than that of **I** due to different molecule shapes between **VII** and **I**. MD simulations carried out by Miklis et al.[33] showed that four Rose Bengal (**I**) molecules could be in the interior region of PPI-5. Four Rose Bengal molecules were located in each large interior cavity with the single benzene ring pointed out and formed good hydrogen bonds with several of the amide sites. We calculated the van der Waals volume of all seven dye molecules using Cerius^2.[34] Results show that dyes **I** and **VII** have similar volumes, 474.31 and 485.62 Å^3, respectively, but very different shapes. Topologic unmatching between the dendrimer cavity and the shape of the dye **VII** molecule leads to very low affinity of dye **VII** to PPI-5.

The effects of charge and topologic matching in guest−host interactions can also be seen from the adsorption behavior of four anionic dyes (**I**−**IV**) in PPI-5 dendrimers. The large difference in affinity between dyes **I** and **II** and dyes **III** and **IV** is due to the substructures **i** and **ii** (Figure 6b). **I** and **II** have the same three-ring substructure **i** (Figure 6b), which is larger than the substructure **ii** (Figure 6b) in **III** and **IV** due to iodization. Simulation results by Miklis et al.[33] show that the shape of **I** fits the cavity of PPI-5 well. The van der Waals volumes of dyes **I**−**IV** are 474.31, 357.92, 314.26, and 265.39 Å^3, respectively. The smaller molecule size of dye **IV** causes there to be less contact area between **IV** and PPI-5 than between **I** and PPI-5. The better topologic matching between the cavities of PPI-5 dendrimers and the molecule shapes of dyes **I** and **II** makes the affinities of **I** and **II** higher than those of **III** and **VI**. The substructure **i** is a key shape in determining the affinity. Although **I** and **III** (or **II** and **IV**) have the same substructure **iii** (or substructure **iv**), their adsorption behaviors are quite different, indicating that the substructures **iii** and **iv** are less important.

We studied the effect of solution pH value on guest−host interactions in dendrimers. A saturated amount of adsorption for **I** and **IV** in PPI-5 dendrimers is shown in Figure 7 as a function of pH values. Adsorption exhibits a maximum around

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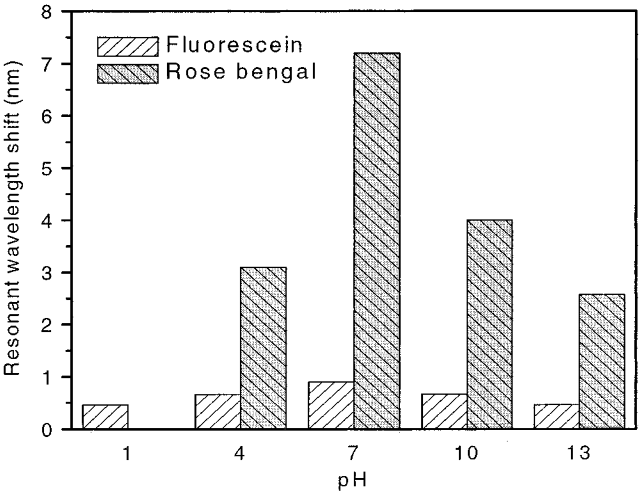

Figure 7. Saturated adsorption of dyes **I** and **IV** as a function of pH value detected by an SPR sensor with a sensor surface modified by PPI-5 dendrimer monolayers. pH values were adjusted by adding 0.1 M NaOH or 0.1 M HCl to 1 M NaCl solution.

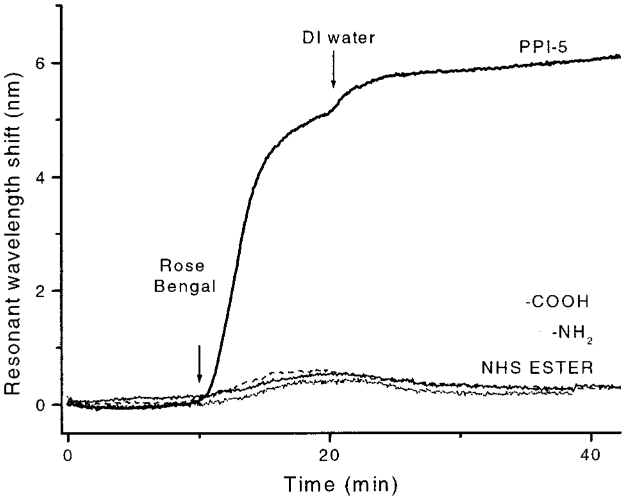

Figure 8. SPR responses to **I** with sensor surfaces covered by PPI-5 dendrimer monolayers, $HSCH_2(CH_2)_{14}COOH$ SAMs, $HSCH_2(CH_2)_{14}COOH$ SAMs activated by NHS, and $HS(CH_2)_2NH_2$ SAMs. The sensor was first stabilized by deionized water for 10 min and then flowed by a dye solution for 20 min, and finally washed by deionized water.

the pH value of 7 for both **I** and **IV**. This behavior is mainly caused by the maximum available number of anions (from dye) and cations (from dendrimer) at this pH value. The adsorption of **I** shows its stronger affinity to PPI-5 dendrimers than that of **IV** at all pH values except 1 due to the better topologic matching between **I** and PPI-5 dendrimer, as discussed above. Undetectable adsorption of **I** in PPI-5 at pH 1 by SPR within a 30 min flow of **I** solution is due to the kinetic effect, as will be discussed in the next paragraph. It should be pointed out that the effect of ion strength on adsorption was minimized through pH values adjusted by adding NaOH or HCl to 1 M NaCl solution, which acts as an ion strength buffer.

The kinetic behavior of dye−dendrimer interactions can be obtained from SPR studies. The effect of the shape of dye molecules on the kinetics behavior can be shown from the response of SPR sensor to dyes adsorbed in and desorbed from PPI-5 dendrimers as a function of time. For example, it took more time for **I** to reach the saturation of adsorption and to diffuse out from dendrimers as compared to **II** (Figure 5), indicating a lower adsorption and desorption rate of **I** than **II**, although the amount of adsorbed **I** is higher than that of **II**. Adsorption and desorption rates also are affected by the pH value of solutions. The time for **I** to reach the saturation of adsorption was 130 min at pH 4, while it was only 30 min at pH 7. The adsorption of **I** was not detectable within a 30 min flow of **I** solutions at pH 1, as shown in Figure 7. This might be related to the change of dendrimer conformation. At low pH values, the interior tertiary amines and exterior amino groups of PPI-5 dendrimers are highly protonated. Strong repulsive force between $-NH_3^+$ leads to the expanded conformation of PPI-5 dendrimers *at the expense of reducing the volume of large cavities*, where dye molecules are adsorbed. We speculate that the lack of adsorption observed at pH 1 might be caused by low diffusion of **I** into the cavities, while the detectable adsorption of **IV** at pH 1 may result from the fact that the van der Waals volume of **IV** is half that of **I**, giving a higher diffusion rate. In general, adsorption and desorption rates of dyes into PPI-5 dendrimers are slow in our experiments compared to those of small VOC molecules.[12,24] The kinetic effect should be taken into account in designing dendrimers for applications in sensors, drug delivery, and catalysis, especially when large guest molecules are involved.

Discussion. To exclude the possibility of strong adsorption of dye molecules on external surfaces of PPI-5 or unreacted carboxyl groups and NHS ester groups on a SAM surface, we performed control experiments in which amino-, carboxyl-, and NHS ester-terminated SAMs were used as SPR sensor surfaces for the adsorption of dyes. Figure 8 shows that there is no strong adsorption of dye **I** on these three surfaces. This suggests that dye **I** molecules should be adsorbed in the interior of PPI-5 dendrimers instead of on the exterior surface of dendrimers where amino groups dominate, or on the SAM surface where unreacted carboxyl groups and ester groups may exist. These results also exclude the possibility of resonant wavelength shifts being caused only by the bulk refractive index change due to the presence of dyes in PBS solutions.

Another set of control experiments was performed for dyes **I**, **V**, **VI**, and **VII** flowing over pure carboxyl-group-terminated SAMs to further test how unreacted carboxyl groups affect the dye−dendrimer interactions. Figure 9 shows that there is a very strong affinity of cationic dyes **VI** and **V** on carboxyl-terminal SAMs due to ionic interactions, while the affinity of anionic dyes **I** and **VII** is very low. From Figure 9 along with Figure 5, we conclude that (1) compact dendrimer monolayer was formed on a pure carboxyl-terminal SAM and blocked unreacted carboxyl groups underneath from access by dye molecules (therefore, dye molecules can only interact with dendrimer cavities) and (2) an SPR sensor responds well when any dye molecule binds to the sensor surface. The very low resonant wavelength shift for cationic dyes **VI** and **V** shown in Figure 5 is due to the very small amount of these dye molecules adsorbed in dendrimers.

There is no direct evidence in our experiments to show how many peripheral amine groups of each dendrimer were attached to the pure carboxyl-group-terminated SAM surface, which could affect both the pK_a profile of dendrimers due to amine groups being converted to amides and the globular structure of

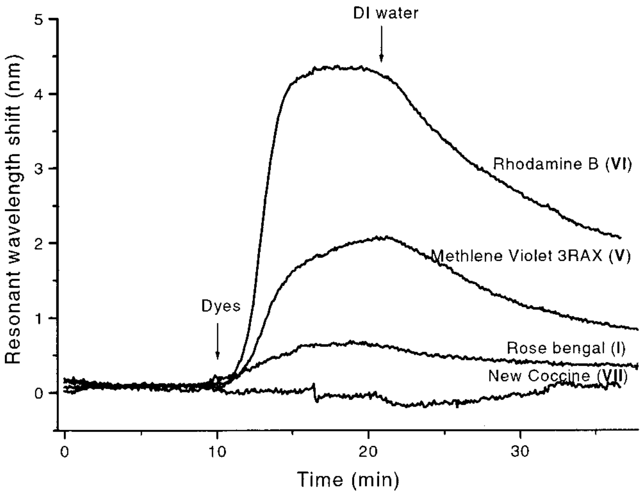

Figure 9. SPR responses to dyes **I**, **V**, **VI**, and **VII** with sensor surfaces covered by $HSCH_2(CH_2)_{14}COOH$ SAMs. The sensor was first stabilized by deionized water for 10 min and then flowed by a dye solution for 12 min, and finally washed by deionized water.

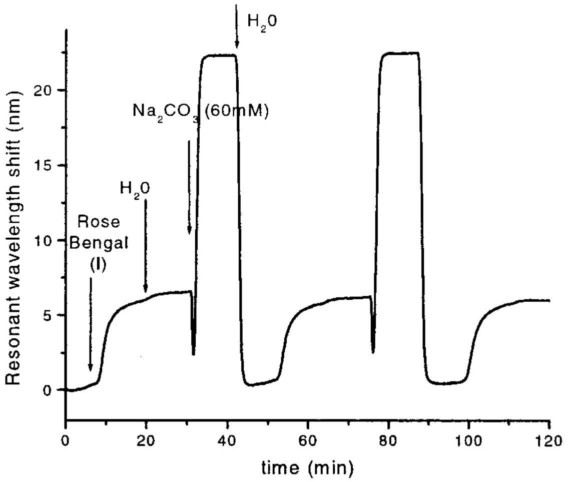

Figure 10. Recycle of an SPR sensor chip modified with PPI-5 dendrimer monolayers. The sensor was stabilized by deionized water for 10 min and then flowed by dye **I** in deionized water for 15 min, and washed by deionized water, 60 mM Na_2CO_3 solution, and deionized water each for 10 min. The same process was then repeated. The highest resonant wavelength shifts were caused by the bulk refractive index of the 60 mM Na_2CO_3 solution.

dendrimer due to dendrimers being confined to a surface. The adsorption of dyes in dendrimers will be affected accordingly. In this work, dendrimer monolayers were prepared in the same way for all dyes studied, and the relative affinity was compared. From the resonance wavelength shift of Rose Bengal adsorbed in dendrimer monolayer (Figure 5) and the simulation result shown in Figure 2, it is estimated that about 70% of the cavities of a PPI-5 dendrimer was occupied by Rose Bengal molecules. This could be the result of lower accessibility to some cavities blocked by substrate and adjacent dendrimers or deformation of dendrimer globular structure.

In addition, regenerability of sensor chips was investigated. Rose Bengal molecules can be washed away from the sensor surface by flowing 60 mM Na_2CO_3 solutions for 10 min, as shown in Figure 10. The affinity of the sensing surface remained the same before and after recycles. Much more dye molecules desorbed from the sensor surface when washed by Na_2CO_3 solutions than when washed by PBS (or other saline solution) and pure water, as shown in Figures 10 and 5, respectively. Although the pH value of washing solutions increases in the order H_2O < PBS < Na_2CO_3, the difference in the desorption of dyes from sensor surface is not simply due to the different pH values of the washing solutions. The desorption was still very low when the surface was washed by 1 M NaCl solution under similar or higher pH values than when washed by 60 mM Na_2CO_3 (results not shown). Such a difference might be caused by the competition between dye and salt anions or/and collapse of dendrimer structure due to bad solvent.

Conclusions

In this work, guest−host interactions between various dye molecules and PPI-5 dendrimers in aqueous solutions were studied using an SPR sensor. A homogeneous PPI-5 dendrimer monolayer covalently bonded on a SAM surface was prepared. The monolayer was stable at a wide range of pH values, ion strengths, and shear forces. The adsorption behavior of various dye molecules having different charges and shapes indicates the importance of match in charge and topology between dye molecules and PPI-5 dendrimers. The pH value of aqueous solution affects not only the amount of dye molecules adsobed in the dendrimers but also the kinetic process. The adsorption and desorption rates of dye molecules depend on the properties of dye molecules and dendrimers as well as solutions used. A fundamental understanding of guest−host interactions in dendrimers and their kinetics is critical to the application of dendrimers, such as in drug delivery, sensing, separation, and catalysis.

Acknowledgment. The authors gratefully acknowledge the National Science Foundation under Grants CTS-0092699 (CAREER Award) and CTS-9983895 for financial support. This work made use of SPR sensors developed under support by the Defense Advanced Research Projects Agency under Grant DAAL01-96-K-3614.

JA0170201