Well-defined assemblies of adamantyl-terminated poly(propylene imine) dendrimers and β-cyclodextrin in water

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Adamantyl-terminated poly(propylene imine) dendrimers **1**–**5** were dissolved in water in the presence of β-cyclodextrin through strong non-covalent interaction between the β-cyclodextrin and adamantyl groups. The solubilization of the dendrimer by β-cyclodextrin is most effective at pH = 2, because at this pH there is complete protonation of the tertiary amino groups present in the dendritic cores, which causes the dendrimers to adopt a stretched conformation. The dendrimers remain in solution at pH ≤ 7, but precipitate under basic conditions, except for **1**, which remains in solution. The stoichiometries of the β-cyclodextrin complexes of **1**–**4** at pH = 2 are **1**·(β-CD)$_4$, **2**·(β-CD)$_8$, **3**·(β-CD)$_{16}$, and **4**·(β-CD)$_{32}$, respectively. For steric reasons not all 64 adamantyl groups of **5** can be complexed by β-cyclodextrin. The stoichiometry of the **5**·(β-CD)$_n$ complex is about 1 : 40 (pH = 2). Steric arguments confirm that the outer surface area of **5** is too small to allow complete coverage by β-cyclodextrins. Qualitative fluorescence measurements at pH = 1 using 8-anilinonaphthalene-1-sulfonate (ANS) as a probe showed that the assemblies of **2**–**5** with β-cyclodextrin act as supramolecular hosts for ANS in water. The binding of ANS is electrostatically driven and increases for higher generations.

Introduction

As part of our strategy to synthesize receptor molecules we have looked at both covalent and non-covalent combinations of different building blocks, such as calix[4]arenes, cavitands, and porphyrins.[1] These receptor molecules are soluble only in organic solvents. However, our ultimate goal is to carry out supramolecular chemistry in water. In relation to this objective we are interested in cyclodextrins and cyclodextrin derivatives as building blocks,[2] or as solubilizing agents for organic hosts.

Amongst others who have used dendrimers as building blocks in aqueous and non-aqueous supramolecular architectures,[3,4] Kaifer *et al.* have used β-cyclodextrin to solubilize cobaltocenyl-terminated poly(propylene imine) dendrimers in water through complexation of the cobaltocenyl units.[5] The solubilization was effective up to the third generation dendrimers (16 cobaltocenyl endgroups). The stoichiometry of the dendrimer·β-cyclodextrin complexes was not reported.

Poly(propylene imine) dendrimers, derivatized with bulky groups like *N*-Boc-protected amino acids or adamantanes, can be considered to be persistent globular structures,[6] obeying the "open core/rigid dense shell" model of the dendritic box as proposed previously.[7] In this model bulky moieties are positioned at the periphery of the dendrimer forming a rigid shell around the propylene imine units, which constitute a relatively open core structure. Their persistent globular structure prevents the dendrimers from folding inside-out, which would lead to aqueous solvation of the relatively hydrophilic cores and shielding of the hydrophobic endgroups, as is the case for poly(propylene imine) dendrimers functionalized with less bulky moieties.[6]

Here we report the formation of well-defined supramolecular assemblies built from β-cyclodextrin and the recently described adamantyl-terminated poly(propylene imine) dendrimers[8] **1**–**5** (Fig. 1) in water. The non-covalent interaction between adamantyl derivatives and β-cyclodextrin in water is generally known to be very strong, owing to the tight fit of the spherical, bulky adamantyl group in the β-cyclodextrin

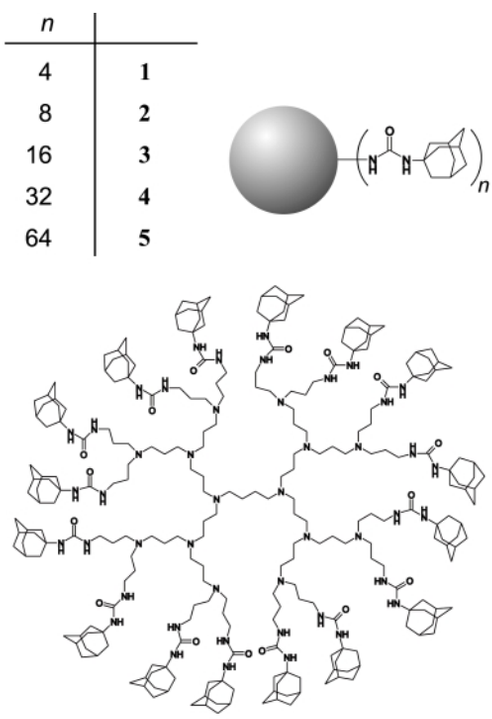

Fig. 1 Adamantyl-modified poly(propylene imine) dendrimers, generations 1–5.

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cavity.[9] Values in the range 10^5–10^6 M^{-1} have been found for the association constants of neutral adamantyl derivatives in β-cyclodextrin.[10] The combination of multiple adamantyl–β-cyclodextrin interactions not only provides a route to well-defined assemblies in water, but also may function as a tool for solubilizing hydrophobic moieties in water. Thus, functional architectures are envisaged that can be rapidly and reversibly transferred from water to organic media with concomitant changing behavior. Moreover, the increase in size of the dendritic endgroups upon complexation with β-cyclodextrin gives us a valuable tool with which to investigate the dendrimer geometries themselves. The stoichiometry of the dendrimer·β-cyclodextrin complexes was determined *via* ^{1}H NMR. Accordingly, the geometry and size of the dendrimer·β-cyclodextrin complexes were studied and compared with the dimensions of β-cyclodextrin in order to theoretically support the NMR data. Finally, the solubilized dendrimers were studied as supramolecular host systems using the fluorescent probes 8-anilinonaphthalene-1-sulfonate and pyrene.

Experimental

Preparation of the dendrimers

Adamantyl-terminated dendrimers **1**–**5**, linked *via* urea moieties, were prepared as described previously.[8]

Dissolution of the dendrimers in water

To the solid adamantyl-terminated poly(propylene imine) dendrimers **1**–**5** (generations 1–5) was added a concentrated (10–12 mM) solution of β-cyclodextrin in H_2O or D_2O ([β-CD]/[adamantyl] > 1). At lower β-cyclodextrin concentrations dissolution of the dendrimer remained incomplete, even when [β-CD]/[adamantyl] ⩾ 1. The pH was adjusted to 2 with aqueous HCl. The mixture was subsequently heated to 60 °C and/or ultrasonicated until a clear solution was obtained (1–4 h). Accordingly, the mixture was diluted with H_2O or D_2O to the stock concentration. After dilution, the pH was checked and lowered to pH = 2 if necessary.

NMR

NMR titrations were carried out at 25 °C using a Varian Inova 300 NMR spectrometer. To solutions of dendrimer in D_2O (0.60, 0.23, 0.11, 0.053, 0.021 mM for **1**–**5**, respectively), having a slight excess of β-cyclodextrin relative to adamantyl endgroups, were added aliquots of a concentrated (12 mM) β-cyclodextrin stock solution in D_2O at pD = 2. The chemical shift of the H3 proton (located at the inside of the cyclodextrin cavity) was monitored as a function of the [adamantyl]/[β-CD] ratio. ^{1}H NMR chemical shifts (300 MHz) are given relative to the H1 signal (4.937 ppm), which is insensitive to guest inclusion due to its location at the β-CD exterior.[11]

Fluorescence measurements

Fluorescence measurements were carried out on Perkin-Elmer LS 50B and Edinburgh FS900 fluorescence spectrophotometers. To 3 mL of a 65 μM solution of adamantyl-terminated poly(propylene imine) dendrimer (**1**–**5**)–β-CD complex ([β-CD]/[adamantyl] = 2) in H_2O at pH = 2 in a cuvette (d = 1.0 cm) was added 30 μL of a 0.97 mM solution of 8-anilinonaphthalene-1-sulfonic acid ammonium salt (ANS) in H_2O ([ANS] = 9.6 μM in cuvette). Subsequently the fluorescence spectrum was recorded, monitoring the emission between 400 nm and 600 nm at an excitation wavelength of 354 nm. After 3 days the fluorescence spectra of the same samples were recorded again.

From a 1.1 μM stock solution of pyrene in H_2O a 12 mM β-CD solution was prepared, which was subsequently used to dissolve **5**. The resultant solution was diluted with 1 μM pyrene solution to a dendrimer concentration of 65 μM, with [β-CD]/[adamantyl] = 2 with pH = 1. Accordingly, the fluorescence spectrum was recorded, monitoring the emission between 350 nm and 600 nm at an excitation wavelength of 340 nm. As a blank, a spectrum was recorded of a solution containing 1.1 μM pyrene and 4.2 mM β-CD (pH = 1).

Results and discussion

Adamantyl-terminated dendrimers **1**–**5** were insoluble in water, even upon protonation of their core amine functionalities.[6] In the presence of a slight excess of β-cyclodextrin relative to the number of adamantyl endgroups, dendrimers **1**–**5** dissolve in water at low pH. After solubilization of the dendrimers, the pH could be raised to 7 while the solutions remained clear. At pH > 7 precipitation of **2**–**5** occurred, while **1** remained in solution even at very high pH.

The above mentioned observations are explained by the complexation of the adamantyl units of the dendrimers in the β-cyclodextrin cavities, driven by hydrophobic interaction. At low pH the dendrimer occupies a fully extended conformation, owing to the repulsive interactions between the protonated tertiary amines.[12] The complete protonation of the cores makes the dendrimers behave like hard spheres with a liquid-like ordering, as is proven by small angle neutron scattering (SANS).[12] The extension renders enough space at the periphery of the dendrimers for all (or most of) the adamantyl groups to be complexed by β-cyclodextrin. Because of the open structure of the protonated dendrimer core, water molecules can penetrate relatively easily, providing good solvation for the positive charges. Upon increase of the pH the amine groups in the dendrimer core become deprotonated,[13] which causes the molecules to collapse due to increase of the hydrophobicity of the core and elimination of the charge repulsion.[12] This collapse reduces the exposure of the adamantyl groups, so that complexation by β-cyclodextrin is hindered, which might result in aggregation and does ultimately lead to precipitation of the dendrimers.

In order to determine the occupancy of the adamantyl-functionalized poly(propylene imine) dendrimers with β-cyclodextrin at low pH, ^{1}H NMR titrations were carried out in D_2O at pH = 2. The chemical shift of the H3 proton, located at the interior of the β-cyclodextrin cavity, was monitored as a function of the [adamantyl]/[β-cyclodextrin] ratio, ρ (Fig. 2). The observed chemical shift, δ, is given by eqn. (1), in which

$$\delta = \delta_f(1 - f_b) + \delta_b f_b \qquad (1)$$

δ_f, δ_b, and f_b are the H3 chemical shifts corresponding to free and bound β-cyclodextrin, and the mole fraction of bound β-cyclodextrin, respectively. The first point in the graph ($\rho = 0$) corresponds to the chemical shift (δ_f = 3.833 ppm) of H3 in uncomplexed β-cyclodextrin. Upon increase of ρ, *i.e.* increase of the dendrimer concentration, δ decreases, indicating the inclusion of adamantyl units in β-cyclodextrin. The maximal change of δ is to the bound value, δ_b, of 3.778 ppm. For **1**, this value was measured directly by preparation of a solution saturated with dendrimer. Fig. 3 shows the ^{1}H NMR spectrum of **1**·(β-CD)$_4$ in D_2O. Integrals showed that ρ = 1,[14] so it can be concluded that **1**·(β-CD)$_4$ was obtained as the sole species.

For **2**–**5**, ρ was kept at values <1 to avoid incomplete dendrimer dissolution. The spectra for **2**–**4** were sharp, as seen for **1** (Fig. 3). All datapoints for **2**–**4** lie on the same line as for **1** (Fig. 2), leading to the same extrapolated bound shift for β-cyclodextrin. This confirms, as expected, that the β-cyclodextrin–adamantyl inclusion is the only interaction between β-cyclodextrin and the dendrimers contributing to the observed β-cyclodextrin chemical shift. It also confirms the quantitative inclusion of all adamantyl units of these generations by β-cyclodextrin. The spectrum for **5** showed line broadening, preventing accurate determination of δ at ρ > 0.6. For **5**, a line

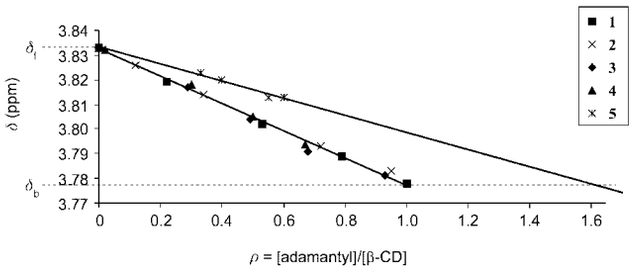

Fig. 2 ^{1}H-NMR titrations of **1**–**5** with β-CD: the H3 chemical shift of β-CD *versus* the [adamantyl]/[β-CD] ratio (D_2O, pD = 2, T = 25 °C).

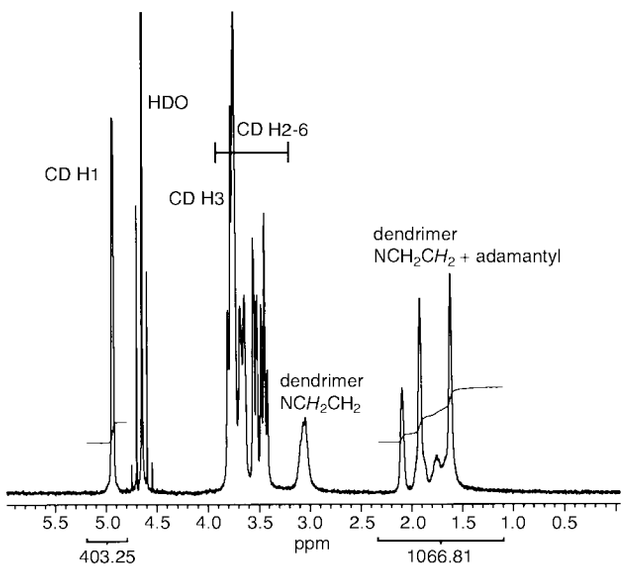

Fig. 3 ^{1}H NMR spectrum of the **1**·(β-CD)$_4$ complex (D_2O, pD = 2, T = 25 °C).

with a significantly smaller slope was obtained (Fig. 2). Based upon the assumption that here also the bound shift, δ_b, is identical to the values for **1**–**4**, this value for **5** is reached at ρ_{max} = 1.61. This indicates that only 62% ($1/\rho_{max}$) of the adamantyl groups are involved in binding, leading to a stoichiometry of **5**·(β-CD)$_n$, where $n \approx 40$. The incomplete occupancy of **5** with β-cyclodextrins is probably due to steric hindrance (see below).

This assumption prompted us to compare the surface areas covered by the β-cyclodextrins and the outer surfaces of **1**–**5**. The latter were derived from size determinations of the primary amine-functionalized parent dendrimers given by Scherrenberg *et al.*[15] using viscosimetry, SANS, and molecular modeling. The adamantyl units are bulky compared to the dendritic core, leading to the persistent globular structure of the dendrimers, but are small compared to β-cyclodextrin. Since the area of the dendrimer outer surface occupied by a single adamantyl unit is about 0.40 nm^2,[6] it is easily seen that, even for **5**, all of the adamantyl groups can reside at the dendrimer outer surface without steric crowding. This is especially true at the low pH employed here, where the branches are fully stretched because of repulsion between the protonated core amines. Only upon inclusion of the adamantyl units in β-cyclodextrin does the area per endgroup at the outer surface increase so much that steric hindrance occurs.

Even the relatively narrow primary side of the conical β-cyclodextrin cavity is wide enough for an adamantyl unit to enter it,[16] so a β-cyclodextrin may approach the dendrimer from either its secondary or primary side and still accommodate an adamantyl group. We therefore used an average value of 1.42 nm for the diameter of the β-cyclodextrin cavity (the diameters of the primary and secondary sides are 1.30 nm and 1.53 nm, respectively, based on modeling studies and on the intra-

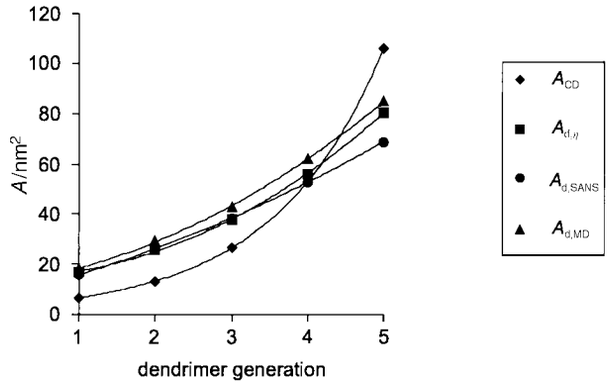

Fig. 4 Dendrimer surface area A_{CD} required for the inclusion of all adamantyl units by β-CD and surface areas inferred from viscosimetric ($A_{d,\eta}$), SANS ($A_{d,SANS}$), and molecular modeling data ($A_{d,MD}$) as a function of dendrimer generation.

molecular distances of β-cyclodextrin as reported by Szejtli[17]). The molecular area per β-cyclodextrin present on a hexagonally packed surface is a_{CD} = 1.65 nm^2.

In order to achieve complete complexation of all adamantyl groups by β-cyclodextrin, the outer surface of the dendrimer (A_d) defined by the centers of the adamantyl units, needs to be larger than the total surface (A_{CD}) occupied by β-cyclodextrin molecules when densely packed, given by eqn. (2), where n is the

$$A_{CD} = na_{CD} \quad (2)$$

number of adamantyl groups per dendrimer. For all five generations, the radius R_d of the dendritic sphere was calculated using eqn. (3), in which r is the geometric radius of the parent

$$R_d = r + r_{ad} \quad (3)$$

primary amine-terminated dendrimer, calculated based on the radii r obtained by Scherrenberg *et al.*[15] *via* viscosimetry (r_η), SANS ($r_{g,SANS}$), and valence consistent force field MD simulations ($r_{g,MD}$). The value for r_{ad}, the radius of an adamantyl urea moiety, was estimated to be about 0.55 nm from a molecular model. The values obtained by SANS and MD are radii of gyration (r_g) and are related to the hydrodynamic radius (r_η) *via* eqn. (4).[15,18] For the large dendrimers

$$r_\eta = r_g\sqrt{(5/3)} \quad (4)$$

studied here, we assume the hydrodynamic radius (r_η) to be equal to the geometric radius (r).

Thus we obtained three sets of approximate surfaces ($A_{d,\eta}$, $A_{d,SANS}$, and $A_{d,MD}$) for the dendritic spheres defined by the centers of the peripheral adamantyl groups of **1**–**5**. Since the viscosimetry and SANS experiments were carried out at pH = 7 (D_2O), the dendrimers did not occupy their fully extended shape due to incomplete protonation of the tertiary amines in the dendrimer core.[13] The MD simulations, however, were carried out assuming a well solvating medium, causing the dendrimers to adopt a stretched conformation, as is the case in our experiments where complete protonation is achieved.

In Fig. 4, A_{CD}, $A_{d,\eta}$, $A_{d,SANS}$, and $A_{d,MD}$ are plotted as a function of dendrimer generation. The radius of a dendrimer increases linearly with generation,[15] which means that dendrimer surface shows a quadratic relationship with the generation number. A_{CD}, however, depends exponentially on the generation number, as does the number of adamantyl endgroups. From a geometrical point of view, the values for $A_{d,MD}$ probably resemble best the surfaces of the extended conformations of **1**–**5** at low pH, while $A_{d,\eta}$ and $A_{d,SANS}$ correspond to the dendrimers in a more collapsed state. Fig. 4 predicts that accommodation of all adamantyl groups by β-cyclodextrin is

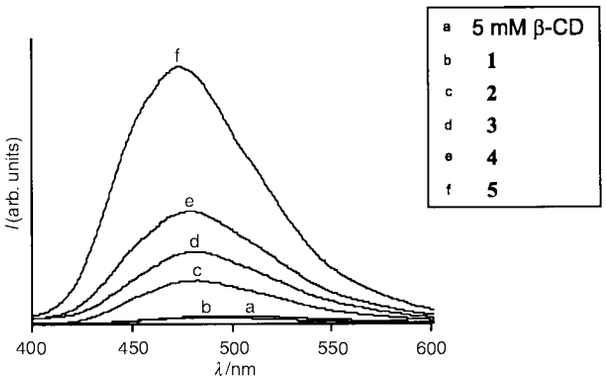

Fig. 5 ANS fluorescence intensities, measured directly after the addition to a β-CD solution with or without 65 μM of dendrimer **1**–**5** (H_2O, pH = 1, T = 25 °C).

sterically possible for all generations, except for generation 5, which is in good agreement with the experimental ^{1}H NMR results. Fig. 4 also suggests that even at pH = 7, up to generation 4 it may still be possible to complex all adamantyl functionalities of a dendrimer. Experimentally, this could not be established because of line broadening of the ^{1}H NMR spectra of the β-cyclodextrin complexes of **2**–**4** at neutral pH. Moreover, this line broadening may even indicate partial decomplexation of β-cyclodextrin due to partial collapse of the dendrimer. As mentioned above, the ^{1}H NMR spectrum of **5**·(β-CD)$_n$ shows line broadening already at pH = 2, further indicating incomplete adamantyl complexation. Most likely, this line broadening is attributed to a relatively slow exchange between free and bound adamantyl units.

The study described above is the first non-covalent example of the sterically induced stoichiometry (SIS) principle, described by Tomalia *et al.* for covalent cases.[19] In this concept, the size of the substituent is compared to the dendritic surface space available per endgroup, of which the latter decreases with increasing generations. Thus, by varying the bulkiness of the reactant, the number of endgroups can be sterically controlled. The so-called "de Gennes dense packed state" is reached when the endgroups exhibit complete steric crowding.[20] Applying this model to the present non-covalent system, this state is reached in the case of the **5**·(β-CD)$_n$ assembly.

Poly(amidoamine) (PAMAM) dendrimers[21] and poly(propylene imine) dendrimers[7,22,23] are known for their abilities to act as hosts for organic analytes in aqueous, as well as organic, media. Dependent on the nature of the terminal functional groups[7,24] and the solvating medium,[12,15] the higher generation poly(propylene imine) dendrimers contain voids in their cores. These dendritic boxes are potential hosts for the complexation of organic dyes.[7] The ability of the β-cyclodextrin complexes of **1**–**5** to act as hosts was studied by fluorescence spectroscopy using the well-known fluorescent probe ANS as a guest for the supramolecular dendritic host system. ANS is a particularly suitable probe, because of its sensitivity towards its micro-environment, *i.e.* the wavelength of the fluorescence maximum is dependent on the polarity of the environment of the dye.[25] We added ANS to solutions of **1**–**5** in water solubilized by β-cyclodextrin at pH = 2. An increase of the fluorescence intensity I, as well as a blue-shift of λ_{max} in the fluorescence of the probe, were observed, except in the case of **1** (Fig. 5). In pure H_2O ANS exhibits only very little fluorescence (λ_{max} = 528 nm) due to quenching by the water OH oscillators. Only a small intensity increase was observed in the presence of β-cyclodextrin alone, due to the weak binding of ANS in this host.[26]

The increase of I upon addition of **2**–**5** strongly indicates that ANS is taken up into the dendrimer, so less contact with water is established, resulting in reduced quenching. The blue-shift of λ_{max} may be explained by a decrease in polarity of the environment of ANS upon complexation. Fig. 5 shows that the increase of I and the blue-shift of λ_{max} increase with dendrimer generation. This indicates that the higher generations shield the ANS probe more effectively from the water than the lower generations, which may imply that the ANS-complexing abilities of the dendrimers increase with each generation.

Titrations to determine binding strength and stoichiometry could not be performed because of slow fluorescence changes occurring once the ANS is complexed by the dendrimer. The immediate increase of I and the decrease of λ_{max} after addition seem to relate to a fast, kinetically driven process. However, after three days the intensity had decreased to a limit of about half the original value, while λ_{max} had red-shifted by approximately 10 nm.

As mentioned above, the incomplete accommodation of the adamantyl groups of **5** may lead to exposed hydrophobic areas of uncomplexed adamantyl groups at the dendritic outer surface. The possibility that this could lead to aggregation was investigated using pyrene as a neutral fluorescent probe. Pyrene is a well-known probe for investigating aggregate formation of amphiphiles in water.[4,27] Inclusion of the probe into the aggregates causes changes in the fluorescence intensity and vibronic fine structure. Also excimer formation is sometimes observed. However, in our case no significant changes in the fluorescence spectrum of pyrene (1 μM) were observed in the presence of 65 μM **5**·(β-CD)$_{40}$ complex, compared to the blank spectrum corresponding to a solution containing only free β-cyclodextrin and pyrene. The lack of significant interaction of pyrene with **5** supports the idea that incomplete adamantyl complexation does not lead to significant hydrophobic areas on the outer surface of the dendrimers with concomitant aggregation.

We attribute the complexation of the negatively charged ANS into the highly positively charged dendrimers to electrostatic interactions only. Hydrophobic forces seem to play no significant role in the binding, since pyrene shows no interaction. This finding is consistent with recent investigations on water soluble primary amine-terminated poly(propylene imine) dendrimers as hosts for the pH-dependent uptake and release of pyrene.[23] Only at high pH, where the interior of the dendrimer is hydrophobic, could the neutral pyrene be complexed.

Conclusions

This study clearly shows the power of the adamantyl–β-cyclodextrin system to solubilize hydrophobic molecules in water. The intrinsically non-water-soluble adamantyl-functionalized poly(propylene imine) dendrimers, **1**–**5**, are effectively solubilized in water at low pH by β-cyclodextrin *via* complexation of the adamantyl units. Well-defined assemblies between cyclodextrins and single dendrimers are formed. In the case of **1**–**4** all adamantyl groups are complexed to β-cyclodextrin. The fact that only about 40 β-cyclodextrins complex to **5** instead of 64 is solely determined by steric reasons and not cooperative weakening of the adamantyl–β-cyclodextrin interaction. Consequently, this work describes the first supramolecular example of sterically induced stoichiometry (SIS).[19] Fluorescence experiments indicate that the remaining uncomplexed adamantyl groups of **5** do not form a hydrophobic area at the dendrimer surface.

The complex stoichiometries of the dendrimer·β-cyclodextrin complexes, determined at low pH, are in good agreement with those theoretically expected, based on calculations concerning dendrimer sizes. At neutral pH, partial decomplexation of β-cyclodextrin may occur due to partial collapse of the incompletely protonated dendrimers. The exposure of hydrophobic sites may subsequently cause aggregation. At higher pH values **2**–**5** precipitate from the solution.

The solubilized dendrimers **2**–**5** act as hosts for ANS at low pH, which we concluded from the changes in the fluorescence

spectrum upon addition of the assemblies to an ANS solution. Since the spectrum of pyrene did not show any significant changes upon addition of dendrimer, the complexation of the negatively charged ANS would seem to be mainly electrostatically driven. The shielding of ANS from the quenching water molecules by the dendrimer becomes more efficient upon increase of dendrimer generation.

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