Poly(propyleneimine) Dendrimers as pH-Sensitive Controlled-Release Systems

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Abstract: Molecular composites were prepared by solubilizing pyrene in diaminobutane poly(propyleneimine) dendrimers having 32 or 64 primary amine end groups (DAB-32 or DAB-64). The dendrimer–pyrene binding constants were determined as $K_{py/DAB\text{-}32} = 16\,725 \pm 200\,\text{M}^{-1}$ and $K_{py/DAB\text{-}64} = 33\,858 \pm 663\,\text{M}^{-1}$ by fluorescence spectroscopy. Fluorescence studies were also employed to probe the release of pyrene from the interior of dendrimers as a function of pH. When the pH value of the system was decreased from pH 11 by addition of HCl, the fluorescence intensity of the system was found to increase by approximately tenfold at pH 2–4. In addition, at pH 2, the ratio of the first to the third vibrational peak of pyrene (I_1/I_3) increased from 0.9, the value typical for pyrene solvated in dendrimer solution, to 1.60, the value characteristic of pyrene in water. Pyrene release from the interior of dendrimers was confirmed by fluorescence quenching when sodium iodide was added, since NaI does not affect the emission of dendrimer-solubilized pyrene. Finally, fluorescence quenching was used to locate the solubilization sites of pyrene within the dendrimer microcavities. These sites are close to the core of the dendrimer, near the tertiary amino groups which are also responsible for quenching the fluorescence of the dendrimer-bound pyrene.

Keywords: controlled release · dendrimers · fluorescence spectroscopy · host–guest chemistry · pyrene

Introduction

Dendrimers are highly branched oligomers or polymers with well-defined structures which have been extensively studied and reviewed[1] in recent years. A dendrimer is characterized by three structural features: i) the central core from which the polymeric branches emanate, ii) the nature of the repeat unit which determines the microenvironment of the interior and in turn the solubilization ability of the dendrimer, and iii) the nature and number of the terminal functional groups which are mainly responsible for the behavior of dendrimers in solution. For instance, the hydrophobic interior of a dendrimer solvates lipophilic compounds, while the water-soluble external functional groups render the resulting molecular composite water-soluble. Following intensive studies of synthesis and characterization of a wide range of dendrimeric compounds,[1] investigations are now focusing on functionalization of the surface groups[2–7] and structural modification of the core,[8] and on solubilization phenomena, based on the concept of the dendritic box.[9–11] According to this concept, dendrimers are viewed as stabilized micellar systems, each oligomer or polymer constituting a micelle in which organic molecules can be solubilized.[12–18]

In the framework of our studies on drug delivery systems involving dendrimers we are now examining a model system based on diaminobutane poly(propyleneimine) dendrimers having 32 or 64 primary amine end groups (DAB-32 or DAB-64), in the hydrophobic interior of which pyrene was solubilized. Efficient incorporation of the active molecule and its controlled release are prerequisites for effective drug delivery systems. With this in mind, we investigated whether changing the pH in the environment of the dendrimers of our model system can affect their solubilizing ability, thus triggering the release of solubilized molecules. The dendrimers in question possess tertiary amine repeating units and are therefore susceptible to protonation, which will certainly modify the environment within the microcavities, favoring in turn the solubilization of more polar compounds.

Results and Discussion

The limiting solubilization of pyrene, measured by absorption spectroscopy, was $1.7 \times 10^{-5}\,\text{M}$ in a $2.3 \times 10^{-3}\,\text{M}$ aqueous solution of DAB-32, and $3.3 \times 10^{-5}\,\text{M}$ in $1.15 \times 10^{-3}\,\text{M}$ of DAB-64. It should be noted that these limiting solubilities of pyrene in the two dendrimers are proportional to the

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corresponding molecular weights of the dendrimers: $[Py_{DAB\text{-}64}]/[Py_{DAB\text{-}32}] = 3.3 \times 10^{-5}/1.7 \times 10^{-5} = 1.94$ and $MW_{DAB\text{-}64}/MW_{DAB\text{-}32} = 7166/3514 = 2.04$. Similar behavior has been reported for PAMAM dendrimers.[18]

In a titration-like addition of DAB-32 or DAB-64 to an aqueous solution containing 6.7×10^{-7} M pyrene (a concentration lower than the solubility of this fluorophore in water, 8×10^{-7} M) a strong quenching of fluorescence intensity of pyrene was observed, as shown in Figure 1. When ethanol was used as solvent instead of water, fluorescence quenching was not observed. In a dendrimer/ethanol solution pyrene can dissolve in the bulk phase and does not need to enter the dendrimer microcavities. The decrease of fluorescence intensity shown in Figure 1 must therefore be attributed to the association of pyrene with the dendrimers and not simply to pyrene–dendrimer quenching collisions. This conclusion is also supported by the following observation: When ethylenediamine, which bears the same terminal NH_2 functional groups as the dendrimers, was added to an aqueous solution of pyrene, the fluorescence was not quenched. In contrast triethanolamine (TEA), which structurally resembles the branching moiety (the tertiary N) of DAB-32 and DAB-64 dendrimers, caused significant quenching ($k_q = 2.0 \times 10^9$ M^{-1}s^{-1}) of pyrene fluorescence in water. These observations suggest that solubilized pyrene molecules reside inside the dendrimer microcavities close to the tertiary amino groups, which are evidently responsible for fluorescence quenching. An alternative explanation, namely that the quenching could be due to the high pH (pH 11) of the dendrimeric aqueous solutions, was eliminated by adding sodium hydroxide (up to pH 11) to an aqueous solution of pyrene, upon which the fluorescence intensity remained constant.

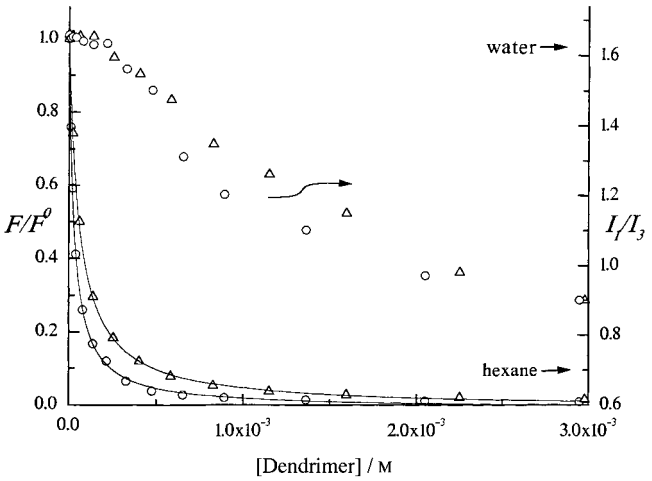

Figure 1. Plot of F/F^0 and of the ratio I_1/I_3 of the pyrene fluorescence plotted against total dendrimer concentration. F^0 is the initial fluorescence intensity of an aqueous pyrene solution (6.7×10^{-7} M) before the addition of dendrimer, and F is the intensity during the titration procedure: △ DAB-32, ○ DAB-64. The lines through the experimental points (F/F^0 vs. [DAB]) are the best fits obtained according to Equation (1).

A quantitative description of the association of pyrene with DAB-32 and DAB-64 was attained by determining the pyrene–dendrimer binding constants $K_{py/DAB\text{-}32}$ and $K_{py/DAB\text{-}64}$. Using the data from Figure 1 and a fitting equation [Eq. (1)] for an assumed 1/1 pyrene/dendrimer complexation,[19] we found the above binding (equilibrium) constants

$$F/F^0 = 1 + \{(F^b/F^0) - 1\}K[D]/(1 + K[D]) \quad (1)$$

to have the magnitudes $K_{py/DAB\text{-}32} = 16\,725 \pm 200$ M^{-1} ($R^2 = 0.9999$) and $K_{py/DAB\text{-}64} = 33\,858 \pm 663$ M^{-1} ($R^2 = 0.9998$). The ratio of these binding constants is 2.02, that is, approximately equal to the ratio of the molecular weights of the corresponding dendrimers, and also to the ratio of the concentrations of pyrene solubilized in the two dendrimers; in other words, $K_{py/DAB\text{-}64}/K_{py/DAB\text{-}32} = [Py_{DAB\text{-}64}]/[Py_{DAB\text{-}32}] = MW_{DAB\text{-}64}/MW_{DAB\text{-}32}$.

Another question relevant to the solubilization properties of the dendrimers is the hydrophobicity of their cavities. Figure 1 shows the variation of the ratio of the first to the third vibrational peak of pyrene (I_1/I_3) as a function of dendrimer added to the original 6.7×10^{-7} M aqueous solution of pyrene. As the titration proceeds, I_1/I_3 decreases to a value of approximately 0.90. This indicates that the microenvironment in the dendrimeric microcavities is considerably less polar than the microenvironment of the bulk aqueous phase, where the ratio has an approximate value of 1.60. In conclusion, in aqueous media pyrene is solubilized within poly(propyleneimine) dendrimers at sites of low polarity and in amounts proportional to the molecular weight of the dendrimer.

Abstract in Greek:

Μοριακά σύνθετα υλικά παρασκευάζονται με διαλυτοποίηση του πυρενίου σε δενδριμερή πολυ(προπυλενο-ιμίνης) τα οποία φέρουν 32 ή 64 πρωτοταγείς ακραίες αμινομάδες (DAB-32 ή DAB-64). Οι σταθερές σύνδεσης του δενδριμερούς με το πυρένιο προσδιορίζονται με φθορισμομετρία και βρίσκονται ίσες με $K_{\pi\upsilon\rho\text{-}DAB\text{-}32}$ = 16725±200 M^{-1} και $K_{\pi\upsilon\rho\text{-}DAB\text{-}64}$ = 33858±663 M^{-1}. Χρησιμοποιούνται μελέτες φθορισμού για τη διερεύνηση της αποδέσμευσης του πυρενίου από το εσωτερικό του δενδριμερούς ως συνάρτηση του *pH*. Καθώς μειώνεται το *pH* με προσθήκη HCl μέχρι την περιοχή *pH*=2-4, αυξάνεται η ένταση του φθορισμού επί δέκα περίπου φορές. Ταυτόχρονα ο λόγος I_1/I_3 αυξάνεται από 0.9, που είναι η τιμή του λόγου όταν το πυρένιο είναι διαλυμένο σε δενδριμερή, σε 1.60 που αντιστοιχεί σε πυρένιο διαλυμένο σε νερό. Η αποδέσμευση του πυρενίου από το εσωτερικό του δενδριμερούς επιβεβαιώνεται επίσης με πειράματα αποσβέσεως φθορισμού χρησιμοποιώντας το ιωδιούχο νάτριο ως υδατοδιαλυτό αποσβέστη. Η ένωση αυτή δεν επηρεάζει το φθορισμό του πυρενίου το οποίο βρίσκεται διαλυτοποιημένο μέσα στα δενδριμερή. Η απόσβεση του φθορισμού χρησιμοποιείται και για τον προσδιορισμό της θέσεως διαλυτοποίησης του πυρενίου στο εσωτερικό των μικροκοιλοτήτων του δενδριμερούς. Αυτές οι περιοχές διαλυτοποιήσεως βρίσκονται πλησίον του πυρήνα του δενδριμερούς, πολύ κοντά δηλαδή στις τριτοταγείς αμινομάδες οι οποίες είναι υπεύθυνες για την απόσβεση του φθορισμού των πυρενίων των συνδεδεμένων με το δενδριμερές. Εν κατακλείδι, η φθορισμομετρία, σε συνδυασμό με φασματοσκοπία απορροφήσεως, επιβεβαιώνουν ότι η αποδέσμευση του πυρενίου από το εσωτερικό του δενδριμερούς προάγεται σε όξινο μέσον, ενώ η εισαγωγή του στο εσωτερικό ευνοείται σε βασικό περιβάλλον.

The association and release of pyrene from the DAB-32 and DAB-64 dendrimers in water as a function of pH was further investigated by monitoring the fluorescence intensity and the ratio I_1/I_3.[20] Figure 2 shows graphs of fluorescence intensity against pH. Hydrochloric acid was added to aqueous solutions

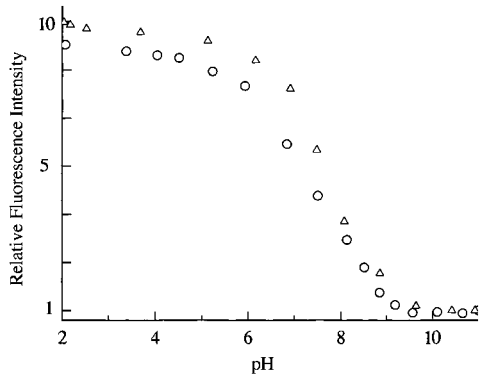

Figure 2. Fluorescence intensity of pyrene plotted against pH of the solvent. [Py] = 3.66×10^{-7} M in water. △ [DAB-32] = 1.00×10^{-3} M; ○ [DAB-64] = 1.00×10^{-3} M.

(pH 11) containing 3.66×10^{-7} M pyrene and 1.0×10^{-3} M dendrimer until the pH had fallen to 2. It can be seen that the fluorescence intensity of pyrene increases by approximately tenfold at pH 2–4. The ratio I_1/I_3 also increases from its initial value of 0.90, characteristic for pyrene solubilized in dendrimers, to a value of 1.60 at pH 2. These results demonstrate that the effect of adding hydrochloric acid is, on the one hand, to eliminate the quenching of pyrene fluorescence, and on the other, to place pyrene in an environment that resembles the aqueous environment, as suggested by the value of the ratio $I_1/I_3 = 1.60$. Two alternative rationalizations of the above findings are plausible. According to the first, hydrochloric acid protonates the quencher, namely the tertiary N of the amino group, thereby destroying its quenching ability. This explains the observed rise in pyrene fluorescence intensity. However, in order to explain the value 1.60 of the I_1/I_3 ratio, one must assume that the protonation of N creates an environment identical to that of bulk water inside the dendrimer microcavity where pyrene resides. In the second alternative, hydrochloric acid again protonates the N center but now, owing to protonation and ensuing increased polarity, pyrene is forced out of the dendrimer into the surrounding water where it fluoresces strongly and naturally exhibits an I_1/I_3 ratio characteristic of pyrene in aqueous solution.

In order to differentiate between these two alternatives, pyrene was solubilized (1.6×10^{-5} M) in an aqueous solution of DAB-32 (2.3×10^{-3} M) and the absorption and fluorescence spectra of pyrene were recorded (Figure 3). Note that this concentration of pyrene is nearly 20 times higher than its solubility in pure water (8×10^{-7} M). When the solution was titrated with hydrochloric acid, the optical density decreased while the fluorescence intensity increased, until the dotted curves of Figure 3 were obtained at pH 4. Figure 3 is interpreted as follows: As hydrochloric acid is added to the dendrimer pyrene solution, the tertiary amino groups are protonated. Pyrene molecules leave the dendrimer and enter the surrounding water, which, however, can only accommodate pyrene up to a concentration of 8×10^{-7} M. Crystallization therefore ensues and pyrene is thus removed from the solution, causing the observed decrease in optical density. The tiny crystals formed cause strong scattering in the absorption spectra and strong excimer emission in the fluorescence spectra, as clearly illustrated in Figure 3. Figure 3 also shows that the optical density at $\lambda = 334$ nm, after subtraction of the background due to the scattering, is 0.03, which corresponds to a pyrene concentration of 7.85×10^{-7} M ($\varepsilon = 3.82 \times 10^4$ cm^{-1}M^{-1}), very close to the water solubility of pyrene. It is also worth mentioning that the absorption spectrum of pyrene before addition of hydrochloric acid, when pyrene is dissolved in dendrimer (solid line in Figure 3), is blueshifted by approximately 440 cm^{-1} with respect to the spectrum at pH 2, when pyrene has entered the surrounding water (dotted line in Figure 3). Comparison of absorption spectra of pyrene in aqueous dendrimer solutions and in pure water yield the same blueshift as described above. This confirms that pyrene is indeed in an environment of pure water at pH 2.

A final confirmation that pyrene enters the aqueous phase upon addition of hydrochloric acid, and that it does not simply remain inside the dendrimer microcavities next to protonated amino groups thus inactivated with respect to their quenching ability, was obtained by means of the water-soluble quencher sodium iodide. As described above, addition of DAB-32 ($2.3 \times 10^{-}$ M) to an aqueous pyrene solution ([Py] = 3×10^{-7} M) greatly reduces the fluorescence intensity, and when 32 μL of 10 N aqueous hydrochloric acid was added to 3 mL of this solution to attain pH 2, the fluorescence intensity recovered, as expected. Finally, when sodium iodide

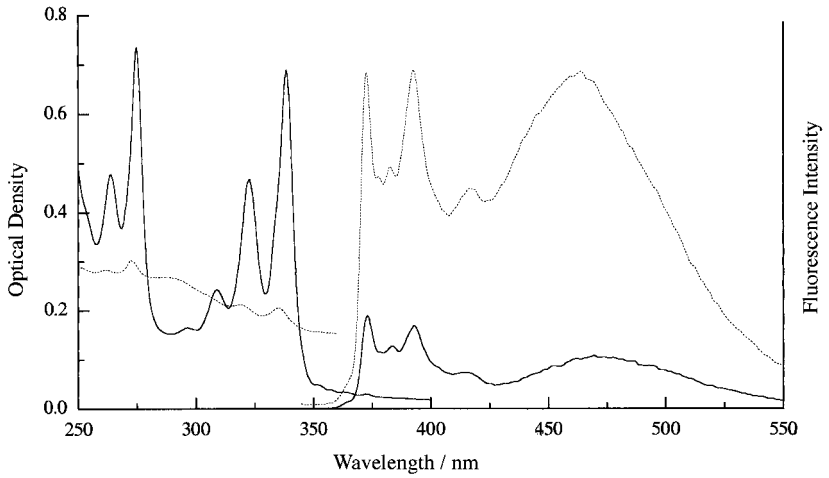

Figure 3. Absorption and fluorescence spectra of 2.3×10^{-3} M DAB-32 with 1.6×10^{-5} M pyrene at pH 11 (solid lines) and pH 2 (dotted lines).

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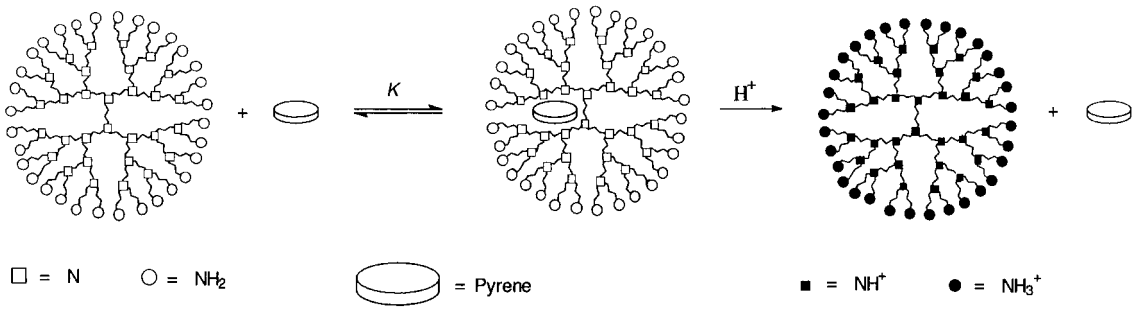

Scheme 1. Schematic representation of the incorporation of pyrene inside the dendrimer and its release in acidic media.

([NaI] = 5×10^{-3} M) was added to the solution, the fluorescence intensity dropped again. In contrast to this result, we found that sodium iodide does not quench the fluorescence of pyrene solubilized in dendrimer. This means that sodium iodide does not enter the interior of the dendrimers but remains in the aqueous phase. Upon addition of hydrochloric acid, pyrene is released into the aqueous phase and sodium iodide quenches its fluorescence. The incorporation of pyrene into the dendrimers and its release in acidic media is shown schematically in Scheme 1.

The data in Figure 2, in combination with reported NMR studies,[21] also allow some insight concerning the site of solubilization of pyrene molecules in the dendrimers. It has been reported[21] that at low [H^+], pH > 10, all the amino groups of DAB-32 and DAB-64 in water are only partly protonated. As the pH value of the solution is lowered by addition of hydrochloric acid, the outer amino groups are the first to be fully protonated at approximately pH 9. As the pH value is further reduced, the N-branching points of the amino groups deep in the core of the dendrimer start to undergo protonation, which is fully completed at about pH 6. Finally, the nitrogen atoms located between the core and the outer surface complete their protonation at even lower pH values. In our experiments no significant increase in fluorescence intensity was observed down to pH 9 (see Figure 2). According to the NMR data the outer tertiary nitrogen atoms are completely protonated at pH 9, and we therefore conclude that pyrene probe molecules do not dissolve in the outer sphere of the dendrimers. Further lowering the pH of the solution by addition of hydrochloric acid results in the protonation of the innermost tertiary N groups, which occurs between pH 9 and pH 6.[21] This protonation stage is associated with a sharp increase in fluorescence intensity of the dendrimer-solubilized pyrene (see Figure 2). We therefore conclude that pyrene occupies a position close to the dendrimer core where its fluorescence is quenched by tertiary amino groups. Upon protonation of these groups due to the addition of hydrochloric acid (pH 6–9), the environment becomes sufficiently polar to repel pyrene molecules which are thus released into the bulk aqueous phase.

In conclusion, the molecular composites resulting from the incorporation of pyrene in poly(propyleneimines) are pH-sensitive: the release of pyrene is induced in acidic media and its incorporation is favored in basic environments. The implications of such behavior for the development of controlled drug-release delivery systems is apparent.

Experimental Section

General: The amine-terminated poly(propyleneimine) dendrimers (i.e. DAB-32 and DAB-64) were purchased from DSM Fine Chemicals Company and used as received. Pyrene was purchased from Aldrich and was purified by zone refining. The Lambda-16 spectrophotometer of Perkin–Elmer was used for absorption spectra, while fluorescence spectra were recorded on a Perkin–Elmer LS-50B fluorometer. For pyrene the excitation wavelength was set to 335 nm.

The pyrene solutions, either in pure water or in aqueous dendrimer solutions, were prepared as follows: An aliquot of a concentrated stock solution of pyrene in hexane (10^{-3} M) was transferred into an Erlenmeyer flask and the solvent was evaporated by rotating the flask slowly. The pyrene was thus obtained as a thin film covering the walls of the flask. Pure water or an aqueous dendrimer solution was then added and stirred overnight.

Titration experiments: In a typical run, a dendrimer solution (1×10^{-2} M) in water containing pyrene (6.7×10^{-7} M) was progressively added to water (containing pyrene at the same concentration in order to maintain the same pyrene concentration throughout the titration experiment) and the fluorescence of pyrene together with the ratio I_1/I_3 were monitored.

Acknowledgements

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[19] In Equation (1), employed for the determination of the binding constants K, [D] is the concentration of the free dendrimer in solution while F^0 is the total fluorescence intensity of a pure aqueous pyrene solution, F^b is the total intensity of pyrene when completely bound, and F stands for the intensity of the latter at any moment during the titration procedure.
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