Encapsulating fluorescein using adipic acid self-assembly on the surface of PPI-3 dendrimer†

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A water-soluble self-assembly has been formed by associating adipic acid molecules onto the surface of the third generation poly(propyleneimine) dendrimer and this system has been used to encapsulate fluorescein.

Dendrimers are monodisperse with a highly symmetrical and hyperbranched three-dimensional cascade architecture. Such unique features result in the presence of void spaces (*i.e.* dendritic voids) within the structure of a dendrimer.[1–4] One distinct application of porous dendrimers is to encapsulate small guest molecules,[5] which can be used as drug delivery vessels,[6–8] fluorescence markers,[9,10] and as recyclable extraction systems,[11,12] *etc*. The multifunctional periphery of a dendrimer also leads to opportunities for versatile modifications.[13–15] An inverse micelle has been generated *via* electrostatic interaction by self-assembling monobasic acids onto the periphery of a dendrimer.[16] However, little work has been done on the self-assembly of dibasic acids on a dendrimer surface.[17]

In this communication, we describe the synthesis of a water-soluble self-assembly of adipic acid molecules on a third generation poly(propyleneimine) (*i.e.* PPI-3) dendrimer surface and the encapsulation of fluorescein within the voids of the adipic acid/PPI-3 self-assembly (Scheme 1). UV-Vis spectroscopy was used to monitor changes in the absorbance of the dye upon encapsulation. Fluorescence spectroscopy was used to detect fluorescent emission change of the dye. Noticeable changes in NMR chemical shifts of the dye also occur as a consequence of encapsulation. By measuring the spin–lattice relaxation time (T_1) and nuclear Overhauser effect (NOE) interactions between the dye and the adipic acid/PPI-3 self-assembly, more detailed information was revealed about the effects of encapsulation on the dye.

Interestingly, the sample of fluorescein alone in solution was yellow-green, while the sample of fluorescein mixed with the adipic acid/PPI-3 self-assembly was red-orange. Upon exposure to UV light, the fluorescein alone solution fluoresced yellow-green; however, the solution of fluorescein in the self-assembled system did not fluoresce at all, which indicated that the fluorescence of fluorescein was severely quenched while encapsulated in the self-assembly of adipic acid/PPI-3.

The UV-Vis spectrum of fluorescein alone has a λ_{max} of 490 nm. Addition of PPI-3 resulted in a red shift to 492 nm. A further red shift to 498 nm was observed when adipic acid was continuously added (Fig. 1a). The final pH values of the systems ranged from 7.5 to 8.0. Over this pH range, the dianion of fluorescein is the prevalent species.[18] The red shift in these UV-Vis spectra of fluorescein suggested that there was an increase in the conjugation of the dye structure upon encapsulation. This conclusion was confirmed from a control study of fluorescein mixed with ethylene diamine and adipic acid (Fig. 1b) in order to rule out the pH effect. Upon addition of ethylene diamine (no encapsulation), there was no change in the peak shape or λ_{max} for fluorescein (λ_{max} = 490 nm). Addition of adipic acid caused a significant blue shift in λ_{max}, due to the reduction of pH upon addition of adipic acid, which brings about protonation of the phenoxide of fluorescein to generate the phenol form.

UV-Vis spectra of fluorescein with PPI-3 and adipic acid at different pH values (Fig. 2) were obtained to gain information about the pH effect on the encapsulation of dyes within PPI-3/adipic acid self-assemblies. At pH = 4.14, the UV-Vis peak is broad with a λ_{max} around 480 nm; and at pH = 6.03, λ_{max} = 496 nm. At the pH values of 6.93, 8.00, and 9.08, λ_{max} = 498 nm, a typical position for encapsulated fluorescein within the PPI-3/adipic acid self-assembly. Changes in intensity of absorbance were observed, with a maximum value when pH = 8.00. A further increase of pH to 9.98 caused a blue shift of λ_{max} to 494 nm. These results indicated that over the pH range of 6.00–9.00, the self-assembly is quite stable, with fluorescein

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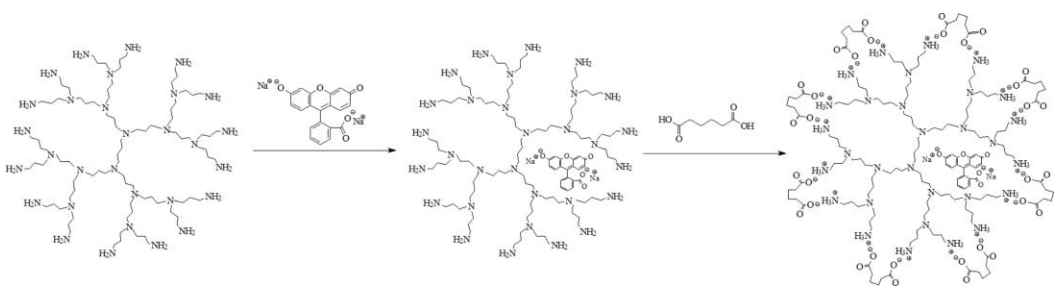


Scheme 1 Encapsulation of fluorescein using the adipic acid/PPI-3 self-assembly.

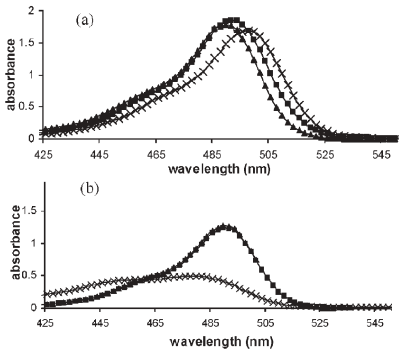


Fig. 1 (a) UV-Vis spectra of fluorescein alone (▲), plus PPI-3 (■), plus adipic acid (×); (b) UV-Vis spectra of fluorescein alone (▲), plus ethylene diamine (■), plus adipic acid (×).

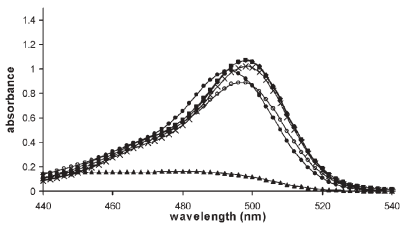


Fig. 2 UV-Vis spectra of fluorescein with PPI-3 and adipic acid at pH 4.14 (▲), pH 6.03 (○), pH 6.93 (♦), pH 8.00 (■), pH 9.08 (×), pH 9.98 (●).

trapped inside. These results also indicate that the dye molecules which sit inside the self-assembly are still be able to 'feel' the different pH environments, which only affect the absorbance of the dye. At extremely high or low pH, the self-assembly can be broken, with fluorescein able to migrate in and out of the dendritic voids.

A fluorescence spectroscopy study also revealed, for fluorescein, that there was a decrease in the fluorescence emission upon addition of PPI-3 and a further reduction with the addition of adipic acid (Fig. 3a). Fluorescence spectra of fluorescein were also obtained in the presence of ethylene diamine (Fig. 3b). The reduction in fluorescence was much smaller than that for fluorescein with the adipic acid/PPI-3 self-assembly. Amines are excellent fluorescence quenchers.[19] Encapsulated within the dendrimer or the dendrimer moiety of the self-assembly, dye molecules are tightly surrounded by the amino groups of PPI-3, therefore quenching is quite efficient. The structure of self-assembly can 'lock' the dye molecules inside the dendrimer moiety more efficiently. For ethylene diamine, quenching depends only on the random encounter of dye molecules with ethylene diamine molecules.

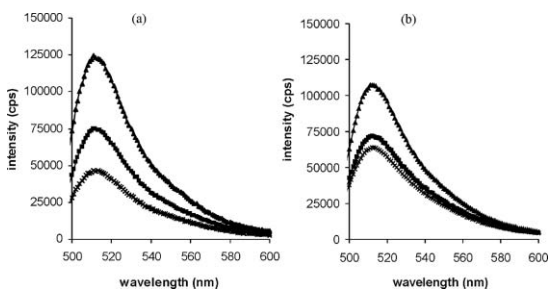


Fig. 3 (a) Fluorescence emission spectra of fluorescein alone (▲), plus PPI-3 (■), plus PPI-3 and adipic acid (×); (b) fluorescence emission spectra of fluorescein alone (▲), plus ethylene diamine (■), plus PPI-3 and adipic acid (×).

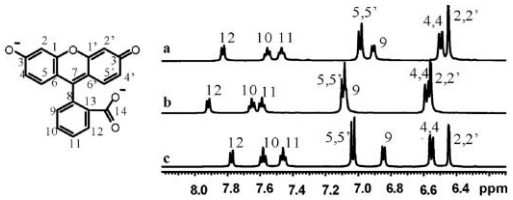


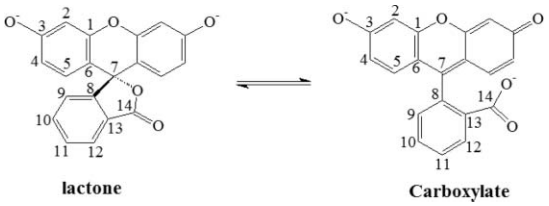
Fig. 4 ^{1}H NMR spectra of fluorescein in D_2O: (a) with adipic acid and PPI-3; (b) with PPI-3; and (c) alone.

NMR spectroscopy was used to glean much information about the encapsulation of fluorescein within the adipic acid/PPI-3 self-assembly and to confirm the results of the UV-Vis and fluorescence studies. Comparing ^{1}H NMR spectra of fluorescein alone and fluorescein in the presence of the PPI-3/adipic acid self-assembly, clear changes in the chemical shift for the resonances of fluorescein (Fig. 4) were observed. These chemical shift assignments are labelled on the spectra. ^{13}C NMR spectra also showed distinct changes in chemical shift.†

These chemical shift changes can be explained by considering changes in the local chemical environment of fluorescein, as well as changes in the overall structure of fluorescein. In the presence of PPI-3, fluorescein shows an affinity towards the relatively non-polar environment of the interior of PPI-3, and migrates into this interior, causing changes in the chemical shift. Addition of adipic acid forms the self-assembly on the surface of the dendrimer and restricts the motion of the dendritic arms. The 'interlocked' self-assembly also reduces exchange of the dye between the open environment and the interior of the dendrimer.

The dianion of fluorescein has two forms in equilibrium: a lactone and a carboxylate (Scheme 2). Both NMR and UV-Vis spectra indicated that the lactone (less conjugated structure) is the predominant form for fluorescein alone. Addition of PPI-3, however, causes changes in the conjugation of the dye, and mixtures of the carboxylate and lactone exist in the system. This can give rise to a red shift in the UV-Vis spectrum since the carboxylate form is more conjugated. There was a distinct downfield shift in the ^{13}C chemical shift for carbon 7 because of a hybridization change from sp^3 to sp^2 upon conversion from the lactone to the carboxylate. A flatter (better conjugation) structure is necessary for fluorescein when the dye migrates into the interior of PPI-3 due to increased steric hindrance of the dye within the interior of the dendrimer.

The ^{13}C T_1 relaxation time was used to confirm the encapsulation of fluorescein and the change in the structure of fluorescein upon encapsulation. Because the transfer of magnetization between different nuclear spins through spin–lattice (T_1) relaxation depends upon the surroundings of the sample molecules (solvent, other sample molecules, *etc.*),[16] comparison of the T_1 relaxation times of the fluorescein alone and with PPI-3 and the



Scheme 2 Equilibrium of different fluorescein structures in solution.

Table 1 ^{13}C T_1 values of fluorescein in different systems

Carbon atoms[a]	^{13}C T_1 relaxation time/s		
	Fluorescein	Fluorescein + PPI-3	Fluorescein + PPI-3 + Adipic Acid
1,1′	2.142	1.106	1.067
2,2′	0.3617	0.2682	0.2933
3,3′	1.437	1.002	0.8101
4,4′	0.3220	0.2440	0.2410
5,5′	0.3119	0.2161	0.2132
6,6′	2.227	1.011	0.6048
7	**0.3119**	**1.002**	**0.6048**
8	1.879	0.7483	0.7288
9	0.3538	0.2522	0.2137
10	0.3237	0.2443	0.2541
11	0.3987	0.2443	0.2397
12	0.3607	0.2345	0.2634
13	2.061	0.9674	1.042
14	4.486	2.730	1.961

[a] The labels for carbon atoms are the same as those in Fig. 4.

PPI-3/adipic acid self-assembly provided information about the interactions of fluorescein with the dendrimer and the self-assembly. All ^{13}C T_1 values for fluorescein are summarized in Table 1. Reductions in T_1 values were observed for most carbon atoms of fluorescein upon addition of PPI-3, due to the encapsulation. However, there was a significant increase in the T_1 relaxation time for carbon atom 7. Carbon 7 in the lactone form (fluorescein alone) is sp^3 hybridized, but upon encapsulation with PPI-3 or self-assembly, the 'flat' carboxylate form existed in which the carbon 7 is sp^2 hybridized, making carbon 7 part of a double bond. This reduces the ability of carbon 7 to transfer magnetization among other atoms, resulting in an increase in T_1 relaxation time. Similarly, for fluorescein in the presence of PPI-3 and adipic acid, there was a reduction of at least 16% in the ^1H T_1 values for all protons, as compared to fluorescein alone.†

2D NOESY (**N**uclear **O**verhauser **E**ffect **S**pectroscop**y**) was used to confirm the presence of fluorescein within the adipic acid/PPI-3 self-assembly. In NOESY, transfer of magnetization among proton nuclei occurs through space (dipole–dipole) interactions of spin systems, aiding in relaxation of the nuclei back to equilibrium.[20] This process is distance-dependent (the larger the space between the nuclei, the less efficient the interaction).[17] The measurement of these interactions can be used to determine if nuclei within a molecule or between different molecules can interact with each other. The 2D NOESY spectrum clearly showed that there were cross-peaks from NOE interactions (Fig. 5) between fluorescein protons (a and a′, b and b′, c and c′, and d) and the interior protons of the PPI-3 moiety in the self-assembled system. This result confirmed that fluorescein was effectively encapsulated within the dendritic voids of the adipic acid/PPI-3 self-assembly.

Results from UV-Vis, fluorescence and NMR spectroscopies clearly demonstrated changes in the dye upon encapsulation. Red shifts of λ_{max} in the UV-Vis spectra of the dye and the quenching of fluorescence indicated that fluorescein was trapped within the void spaces of the PPI-3 moiety of the self-assembly. Changes in ^1H NMR spectra of fluorescein, reduction in the spin–lattice relaxation (T_1) times, and NOE interactions between fluorescein and the self-assembly indicated an efficient encapsulation of the dye inside the PPI-3/adipic acid self-assembly. This water-soluble self-assembly has potential for the encapsulation of water-insoluble drugs in order to enhance their efficacy in the treatment of diseases.[21]

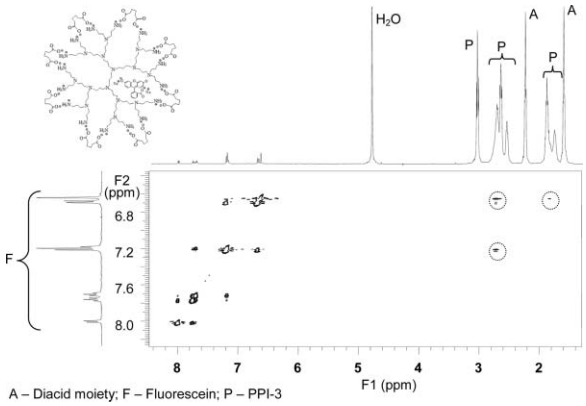


Fig. 5 Expansion of the 2D NOESY spectrum of fluorescein encapsulated in the interior of the PPI-3 moiety of the adipic acid/PPI-3 self-assembly.

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