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NMR and ESR investigations of the interaction between a carboxylic acid and an amine at the focal point of L-lysine based dendritic branches

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This paper reports the characterisation of supramolecular complexes formed between the carboxylic acid group at the focal point of host dendritic branches based on L-lysine building blocks and an amine group on an appropriate guest molecule. ^{1}H NMR titration investigations indicate that the interaction is relatively weak. Interestingly the dendritic generation appears to have no effect on the thermodynamics of benzylamine recognition – in contrast to previous studies in which charged guests have been bound to dendritic hosts. Control experiments using dendritic branches in which the carboxylic acid is protected as a methyl ester indicate that there is only a small amount of non-specific binding of the amine functionalised guest molecule within the dendritic framework itself. ESR investigations clearly show the binding between the dendritic branch and amine functionalised TEMPO radicals. Most interestingly, rotational correlation times can be determined from the ESR studies and they indicate that the mobility of the TEMPO radical is diminished on binding to the dendritic branch. Notably this effect is generation dependent, with larger dendritic branches having a more dramatic effect on the tumbling of the radical. Control experiments clearly prove the importance of the acid-base interaction and also demonstrate that effective binding only occurs in non-polar solvents. These results therefore illustrate that using host–guest chemistry at the focal point of a dendritic structure is an effective way to control and modify the solution phase properties and mobility of active species such as radicals.

Introduction

Much attention has recently focused on discovering the unique properties of dendritic macromolecules.[1] In particular, supramolecular dendrimer chemistry, the combination of dendritic molecules and intermolecular interactions, has become an important theme.[2] In the initial development of dendritic technology, most attention focused on fully formed spherical dendrimers. Such systems can bind guest molecules using binding sites encapsulated at the dendritic core, and hence mimic protein behaviour.[3] A wide range of different recognition motifs have been encapsulated within dendritic structures, including porphyrins,[4] cyclophanes,[5] hydrogen bonding clefts,[6] cyclodextrins,[7] cyclotriveratrylenes[8] and anion binding ferrocene derivatives.[9]

Increasingly, individual dendritic branches (dendrons) are of interest in supramolecular assembly processes – partly because it is possible to more easily synthesise a wide range of dendron structures. The assembly of dendritic branches into superstructures driven by hydrogen bonding or solvophobic effects has been investigated primarily by the groups of Zimmerman[10] and Percec,[11] who have reported hexameric rosettes and liquid crystalline arrays.[12] However, dendrons are also of interest for their ability to bind guest molecules at their focal points using complementary intermolecular interactions. For example, our group,[13] as well as those of Stoddart[14] and Gibson,[15] have reported dendritic branches functionalised at the focal point with crown ether derivatives. These dendritic crown ethers are able to bind appropriate guest molecules such as protonated amines, and there has been much interest in the effects of the dendritic structure on the thermodynamics of the molecular recognition process. In a similar manner, hydrogen bond receptors[16] and metal binding ligands[17] have been attached to the focal point of different dendrons. This general approach can be used to assemble supramolecular spherical dendrimers in which multiple branches are held in place around an appropriate template *via* non-covalent interactions.

We have been particularly interested in the assembly of supramolecular dendrimers as a consequence of interactions between carboxylic acids and amine groups.[18] Although such interactions are known to be weak, even in non-competitive solvents, they are very general in scope. Using such interactions, we have achieved solubilisation of amine-functionalised hydrophilic dyes into apolar media *via* interaction with the carboxylic acid group at the focal point of an L-lysine derived dendritic branch.[19] Specifically, we observed that higher generation dendritic branches were better able to solubilise hydrophilic dyes – we proposed that this was a consequence of more effective encapsulation. Furthermore, using this approach, we were able to transport hydrophilic dyes through an apolar phase and deliver them into an aqueous medium.[20] Acid–amine interactions at the focal point of a dendritic structure also form the basis of our novel two-component supramolecular dendritic gelation system, which we have recently illustrated to have a high degree of tunability.[21]

Given the general interest in the recognition of guest species at an encapsulated binding site, we were interested to learn more about acid–amine interactions and, in particular, the way in which they were controlled by the dendritic structure. To this end, we performed a series of NMR and ESR experiments to monitor the interaction between carboxylic acid functionalised dendritic L-lysine and amine-functionalised probes (benzylamine and 4-amino-TEMPO) (Fig. 1). The results of these studies are reported in this paper and we clearly delineate the effects that dendritic branching has on the binding. In addition, this is the first time that the effect of binding a spin probe at the focal point of individual dendritic branches has been elucidated.

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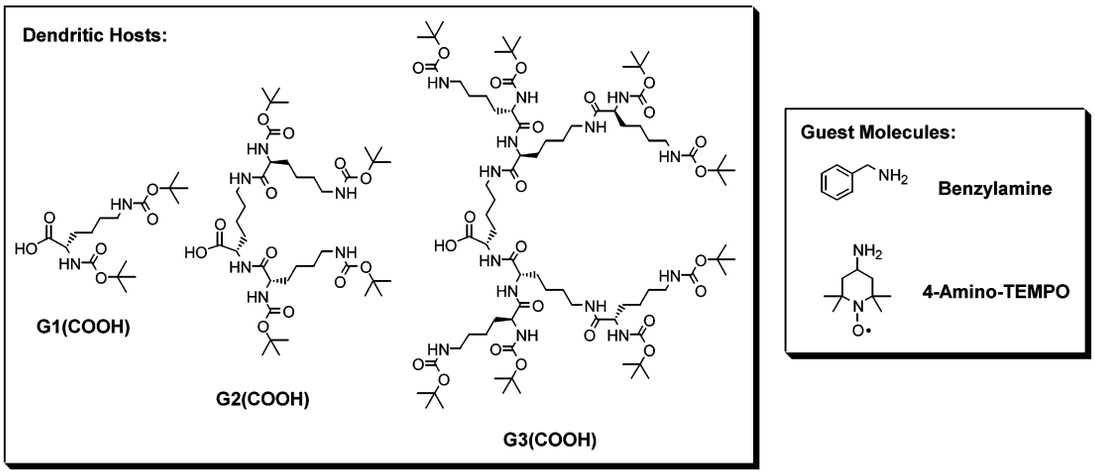

Fig. 1 Dendritic host molecules and amine functionalised guests.

Results and discussion

Synthesis

Dendritic branches **G1(COOH)**, **G2(COOH)** and **G3(COOH)** were synthesised using solution phase methodology previously reported by us, and all data were consistent with the proposed structures.[22] Dendritic branches with the focal point protected in the form of methyl esters **G2(COOMe)** and **G3(COOMe)** were also synthesised using the same well established methodology.[22] Amine functionalised guests, benzylamine and 4-amino-TEMPO, were purchased from standard commercial sources.

NMR investigations

The association between L-lysine based dendrons (**Gn(COOH)**) and benzylamine was investigated using ^{1}H NMR titration methodology. Benzylamine was chosen as the binding partner because it exhibits good solubility in CD_2Cl_2, and the benzylic ^{1}H resonances are close to the proposed binding site and are distinctive (*ca*. 3.82 ppm) – therefore they are easy to monitor in the ^{1}H NMR spectrum during the titration. The titration was performed by sequentially increasing the concentration of the dendritic branch present in solution and monitoring the ^{1}H NMR spectrum of benzylamine. Fast binding was observed, and a time-averaged benzyl peak shifted downfield on the addition of **Gn(COOH)** (Fig. 2). This downfield shift is consistent with the interaction of the amine group with an acidic proton. There is a question-mark over the degree of proton transfer in the complex. Theoretical predictions would indicate that neutral complexes are preferred in apolar solvents, whilst the charged form of the complex is preferred in more polar environments.[23] Dichloromethane has a dielectric constant of 8.93, and should be borderline between the two cases,[23b] and it is therefore not clear whether complete proton transfer from acid to amine occurs (with an electrostatically bound $CO_2^- \cdots {}^+NH_3$ complex being formed) or whether the proton can be considered to remain primarily on the carboxylic acid (with a neutral $CO_2H \cdots NH_2$ hydrogen bond being formed). It is nonetheless certain that the two components remain bound to one another in any case. The ESR results (see below) provide supporting evidence for this interaction between dendron and benzylamine.

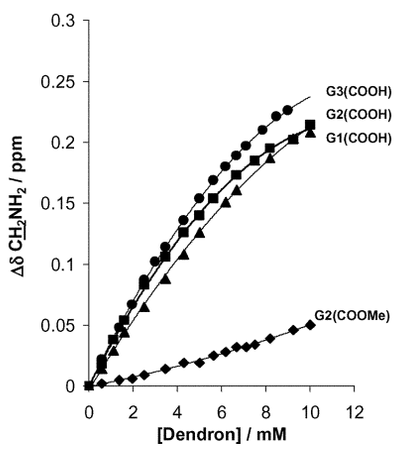

Fig. 2 NMR titration data for benzylamine (2 mM) with the addition of various dendrons, in CD_2Cl_2 solution.

Interestingly, all three generations of dendron induced very similar changes in the NMR spectrum of benzylamine. The binding curves were shallow, indicating weak binding, even in this non-competitive solvent system. We used HYPNMR to calculate binding constants from the titration curves.[24] Log *K* values were determined as 1.9 (± 0.2) for each of **G1(COOH)**, **G2(COOH)** and **G3(COOH)**. These values should, however, be viewed with some caution, as 1 : 1 stoichiometry was assumed in the fitting process and the binding constants do not, therefore, take account of any non specific interactions.

It is noteworthy, that there is no obvious dendritic effect on the binding in this case. This is interesting, as it indicates that the dendritic effects on dye solubilisation we observed previously[19,20] do not stem from enhanced binding of the dye by higher generation dendrons, but rather from enhanced encapsulation and protection from the apolar solvent. This NMR study therefore provides unambiguous confirmation of our previously published hypotheses about the effect of encapsulation on supramolecular dye solubilisation.[19,20] These results also form an interesting contrast with our recent results investigating K^+ binding at the focal point of dendritic crown ethers,[13] and anion binding in ferrocene derivatives,[9] in which the binding strengths were observed to decrease with increasing dendritic functionalisation. These results may therefore indicate there is a fundamental difference between the binding of neutral and charged guests within dendritic superstructures, with the dendritic branching disfavouring the binding of charged species, but not neutral ones.

An important control experiment was performed in which **G3(COOMe)** (the third generation dendritic branch in which the carboxylic acid is protected as a methyl ester) was titrated with benzylamine. A small downfield shift of the benzylic protons was observed. This can be attributed either to general hydrogen bonding and polarity effects, or a small amount of non-specific binding of benzylamine within the polar branched architecture. However, any association was too weak to quantify with an equilibrium constant. This control therefore confirms the predominant importance of interactions between the carboxylic acid and the amine group for achieving effective recognition.

Table 1 Rotational correlation times (τ_c) determined for 4-amino-TEMPO (1.6×10^{-5} M) in the presence of a variety of dendritic additives (3.0×10^{-2} M) in CH_2Cl_2 solution

Solvent	Dendron	τ_c/s
CH_2Cl_2	None	0.1×10^{-10}
CH_2Cl_2	**G1(COOH)**	2.9×10^{-10}
CH_2Cl_2	**G2(COOH)**	2.9×10^{-10}
CH_2Cl_2	**G3(COOH)**	4.9×10^{-10}
CH_2Cl_2	**G2(COOMe)**	0.1×10^{-10}
MeOH	**G2(COOH)**	0.3×10^{-10}

Electron spin resonance (ESR) investigations

Electron spin resonance (ESR) techniques have previously been used to investigate several aspects of dendrimer chemistry. Radicals can either be covalently attached to the dendrimer (a spin *label*) or a separate molecule can be used as a spin *probe*. Spin labels have been attached to the surface of spherical dendrimers, particularly in order to monitor the dendrimers' abilities to interact with DNA.[25] Dendrimers functionalised with spin labels have been used to monitor the dynamic behaviour of the dendritic superstructure.[26] Dendrimers containing ESR active metal ions have also been investigated using ESR methods.[27] Spin probes, on the other hand, have been used to investigate the hydrophobic and hydrophilic binding sites of carboxylate terminated poly(amidoamine) (PAMAM) dendrimers, giving important information on binding at the dendritic surface and insertion of the spin probes into the dendritic superstructure. Ottaviani and co-workers have investigated assemblies comprised of PAMAM dendrimers and cationic surfactants.[28] Using this method, very detailed information was gained about the mutual interactions of the surfactant molecules, the dendrimers and the spin probe. Spin probes have also recently been used to monitor the molecular mobility of organosilicon dendrimers.[29]

Given the wealth of information potentially available, we were interested in exploiting ESR to further characterise our supramolecular approach to dendrimer assembly. The spin probe 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy (4-amino-TEMPO) was chosen to investigate binding at the dendritic focal point of **Gn(COOH)**. In principle, the carboxylic acid at the focal point should bind to the amine group on the spin probe and the probe's association with the dendritic molecule, and its potential encapsulation should be observable in the ESR profile. There have not been any previous reports of the binding of a spin probe at the focal point of individual dendritic branches.

Fig. 3a shows the ESR spectrum of 4-amino-TEMPO in CH_2Cl_2. As expected, three spectral lines are observed due to the spin quantum number for nitrogen being 1. As the probe is free to tumble in solution, all three lines are sharp and have approximately equal intensities. Using the equations developed by Stone *et al*,[30] a quantitative rotational correlation (or tumbling) time (τ_c) could be calculated (Table 1). For 4-amino-TEMPO in CH_2Cl_2, this value is 0.1×10^{-10} s. This rapid tumbling implies that the probe has full mobility in solution. Fig. 3b shows the ESR spectrum of 4-amino-TEMPO in the presence of an excess (*ca.*. 2000 eq.) of **G1(COOH)**. It is notable that a very different spectrum is observed, with the three spectral peaks no longer being of equal height. This effect is typically observed when nitroxide radicals have hindered mobility (for example in viscous solutions), and the spectrum implies that the presence of **G1(COOH)** hinders the mobility of the radical.[30] Figs 3c and 3d illustrate that **G2(COOH)** and **G3(COOH)** respectively have similar effects – although the decrease in peak height for the third peak is considerably greater for **G3(COOH)**. The relative peak heights can be used to calculate the rotational correlation times (Table 1). These have increased compared to the spin probe on its own – in other words the dendritic branches decrease the mobility of the spin probe. Dendrons **G1(COOH)** and **G2(COOH)** have similar effects on the τ_c value (2.9×10^{-10} s). We would argue that the effect of these dendritic branches on the mobility of the spin probe is realised on the molecular scale, with complexation of the dendritic branch to 4-amino-TEMPO *via* acid–base hydrogen bond interactions giving rise to a complex in which the spin probe has reduced mobility. It is noteworthy that **G3(COOH)** has a significantly greater effect on the spin probe's mobility ($\tau_c = 4.9 \times 10^{-10}$ s). The NMR results described above indicate that this is not due to stronger binding by the higher generation dendron. This result therefore indicates a degree of dendritic encapsulation of the spin probe – markedly hindering its mobility. This observation is analogous to the dye uptake studies published previously,[19] in which **G3(COOH)** showed significantly enhanced solubilisation of the dyes when compared with **G1(COOH)** and **G2(COOH)**. This ESR study therefore provides supporting evidence that this larger dendron provides sufficient branching to extend around the carboxylic acid at the focal point, hence offering more effective encapsulation of the bound template molecule.

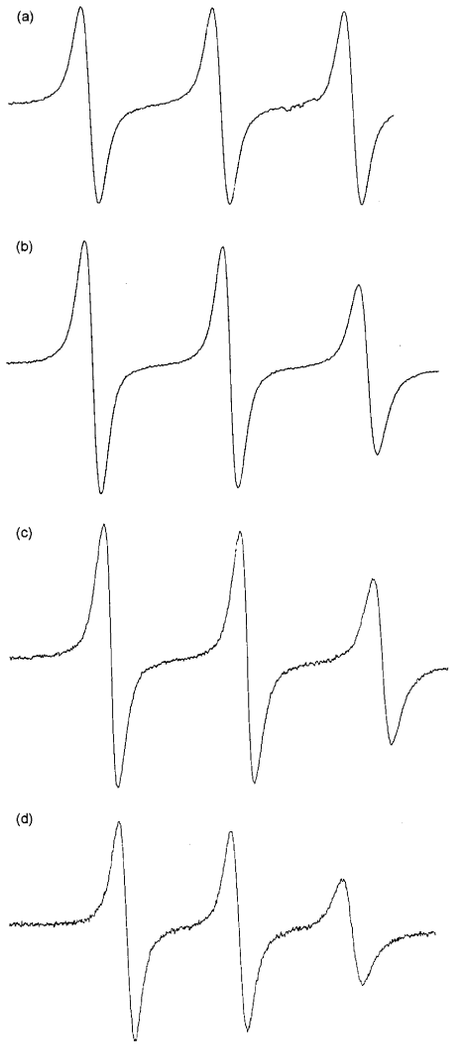

Fig. 3 ESR spectra of 4-amino-TEMPO (1.6×10^{-5} M) in CH_2Cl_2 solution in the presence of (a) no dendron, (b) **G1(COOH)** (3×10^{-2} M), (c) **G2(COOH)** (3×10^{-2} M), (d) **G3(COOH)** (3×10^{-2} M). All spectra are centred around 3365 G, with a peak to peak splitting (hfs) of 15.8 G.

It was important to rule out the role of secondary effects, such as the dendrimer increasing the viscosity of the solution, or interaction of the spin probe with amide groups inherent in the branching itself. To this end, a control experiment was performed in which the spin probe was mixed with excess **G2(COOMe)** and the ESR spectrum determined. The

protected dendron did not restrict the mobility of the probe in any way. Indeed the calculated τ_c value was equivalent to that for 4-amino-TEMPO dissolved in CH_2Cl_2 in the absence of any dendron (0.1×10^{-10} s). This result confirms the essential role played by the acid-amine interactions in mediating the effect of the dendritic branching on the spin probe. Further corroboration of the importance of acid-amine interactions in mediating the mobility of the spin probe was obtained using an unfunctionalised TEMPO radical as spin probe. In all cases, the presence of the dendrons had no effect on the mobility of this spin probe, indicating there was no interaction between the dendrons and the probe in this case.

The role of solvent was investigated by studying the interaction between 4-amino-TEMPO and **G2(COOH)** in methanol. The τ_c value was calculated as 0.3×10^{-10} s, showing that any mobility restriction of the spin probe in this competitive solvent is negligible. We therefore argue that the presence of competitive solvent is sufficient to disrupt the association of dendron and probe in this case. This is not surprising given the low association constant ($\log K = 1.9$) determined in non-competitive CH_2Cl_2 solution by ^{1}H NMR titration methods (see above). We have shown previously, that in order to effectively extend the principles of supramolecular dendrimer construction to more polar solvents (such as methanol) different recognition motifs (such as crown ether $\cdots NH_3^+$ interactions) are preferred.[13]

Conclusions

This paper provides important further characterisation of the supramolecular dendrimers developed by our group which are dependent on interactions between carboxylic acid and amine groups.[19–21] In particular, we have characterised the acid–amine interaction by NMR titration methods and shown that it is, perhaps surprisingly, invariant with dendritic generation. This is in contrast to previous reports in which ionic guests have been bound by dendritic structures,[9,13] and may indicate that different principles apply for the recognition of neutral and charged species by dendritic hosts. We have also performed the first study in which an ESR active spin probe has been bound at the focal point of a dendritic branch and indicated that the dendron reduces the mobility of the spin probe. This effect *is* dendritically controlled. The NMR studies mean that we can be certain this is not simply a consequence of stronger binding to the higher generation dendrons, but must instead reflect a greater degree of encapsulation of the spin probe caused by the larger dendritic host, which hinders the mobility of the radical to a greater degree. Pleasingly, the results presented in this paper are in direct agreement with our previous studies in which we used such acid–amine interactions for the solubilisation and transport of hydrophilic dyes,[19,20] and they add significantly to our understanding of the host–guest recognition processes taking place in this intriguing class of weakly bound supramolecular dendrimers.

Experimental

^{1}H NMR titration experiments were performed using a Bruker AMX-500 spectrometer at 300 K. The concentration of benzylamine was 2 mM throughout, with aliquots of a solution of dendron (15 mM) and benzylamine (2 mM) being added. ESR spectra were recorded on a Jeol RE1X spectrometer with 100 kHz modulation, using X-band frequency. Concentrations were as reported in the results and discussion section.

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