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Short communication

Generation-dependent encapsulation/electrostatic attachment of phenobarbital molecules by poly(amidoamine) dendrimers: Evidence from 2D-NOESY investigations

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Abstract

The interactions of phenobarbital with different generations of amine-terminated poly(amidoamine) (PAMAM) dendrimers were investigated by using two dimensional-nuclear Overhauser effect spectroscopic (2D-NOESY) investigations. The NOESY spectra clearly showed that there were cross-peaks from NOE interactions between the protons of phenobarbital and the protons in interior cavities of generation 5 or generation 6 PAMAM dendrimers but none of such cross-peaks was found in the case of generation 3 or generation 4 dendrimers. The NOESY analysis, together with aqueous solubility data, suggested that higher generation dendrimers are more capable of encapsulating phenobarbital molecules into the interior cavities than lower generation dendrimers, and that lower generation dendrimers are much easier for the electrostatic attachment of phenobarbital molecules than higher ones at a fixed mass concentration.

Keywords: Polyamidoamine; PAMAM; Dendrimer; Encapsulation; Electrostatic attachment; NOESY; Drug delivery

Dendrimers are the fourth major class of macromolecular architectures with featured properties, such as nano-scaled globular shapes, regular and high degree of branching, well-defined number of peripheral functional groups, hydrophobic or hydrophilic cavities in the interior, and extremely low polydispersity [1–7]. During the past decade, dendrimers have proved to be promising candidates in the design of new drug delivery systems [3,8–13] due to the following reasons. First, the interior cavities of dendrimers provide them reasonable choices as unimolecular micelles for encapsulation of guests, especially small drug molecules [8,14]. Second, high density of primary amine groups on the surface of amine-terminated dendrimers endues these dendritic architectures with the capability for electrostatic attachment of negatively charged guests [1,2,15–18]. Last but not least, well-defined peripheral functional groups make it easy for drug molecules, targeting moieties, solubilizing groups, and other functional units to be present on the surface of dendrimers in a multi-valent fashion [19–24]. Although numerous studies on the interactions between dendrimers and hydrophobic drugs have been reported in the literatures [8,25–29], little research has been conducted on the relationships between electrostatic interaction and hydrophobic encapsulation when varying the dendrimer generations.

In this communication, we investigate the host properties of poly(amidoamine) (PAMAM) dendrimers by using two dimensional-nuclear Overhauser effect spectroscopy (2D-NOESY) and aqueous solubility studies. Phenobarbital, a clinically established anti-convulsant drug with extremely low aqueous solubility, is used as a model drug. As shown in Fig. 1a and b, the NOESY spectra exhibit strong cross-peaks between the proton

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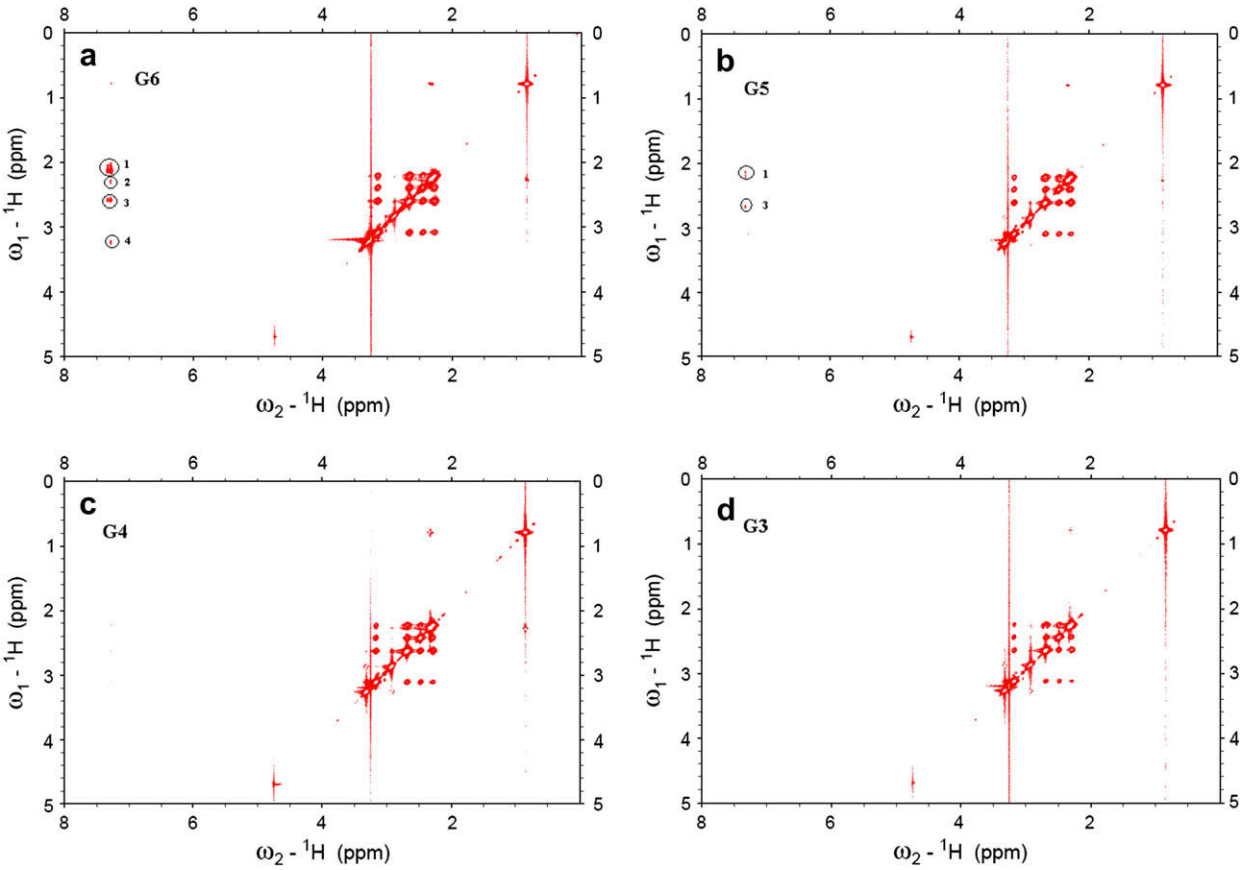
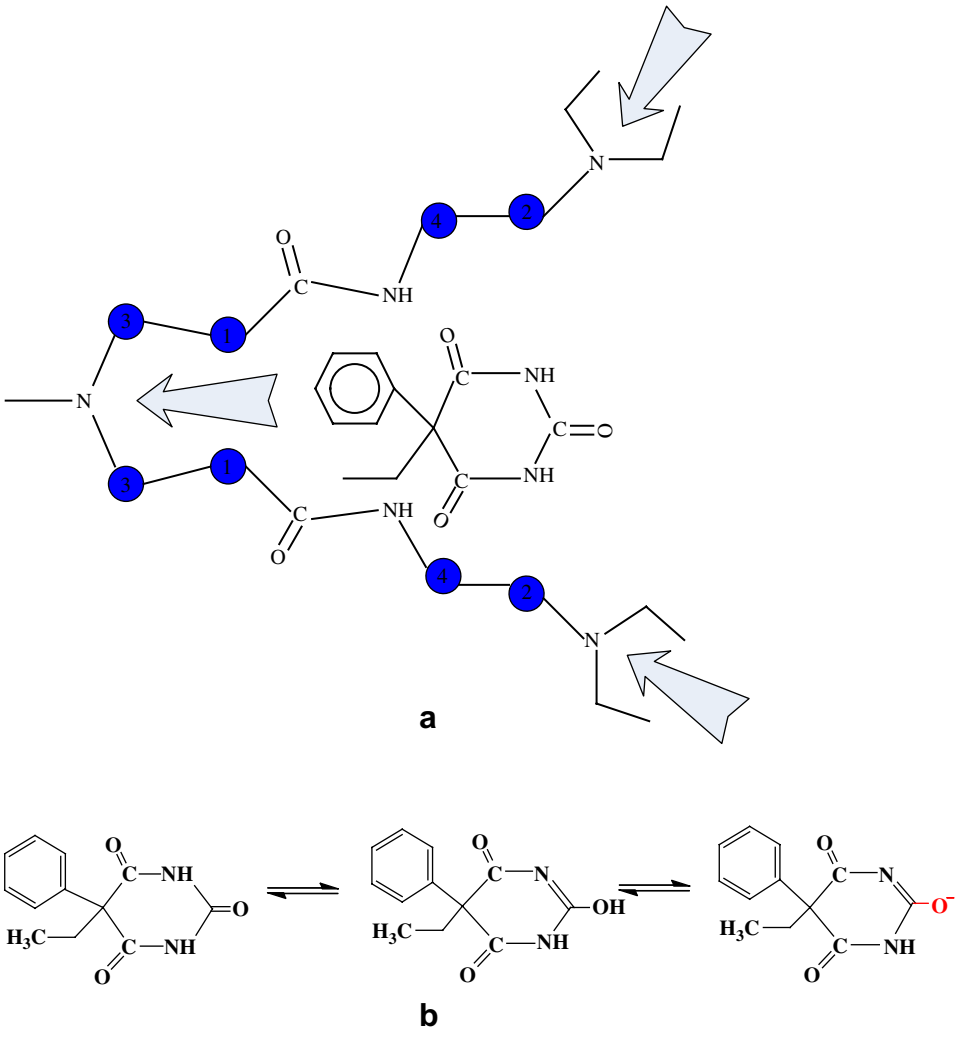


Fig. 1. 2D-NOESY spectra of phenobarbital encapsulated in the cavities of (a) G6, (b) G5, (c) G4, and (d) G3 amine-terminated PAMAM dendrimers.

signals of the aromatic ring of phenobarbital and those of generation 5 (G5) and G6 amine-terminated PAMAM dendrimers. In particular, for the 1-H and 3-H protons of both G5 and G6 PAMAM dendrimers, strong interactions with the aromatic protons of phenobarbital are observed. Nonetheless, no cross-peaks between the 2-H and 4-H protons of dendrimer and aromatic protons of phenobarbital is found for G5 dendrimer, and only weak cross-peaks for such interactions are observed in the case of G6 PAMAM dendrimer. 2D-NOESY experiment measures the distance-dependent NOE effect between protons separated by less than 5 Å, suggesting the larger the space between the protons, the less efficient the interaction [30–32]. Reasonably, the guest molecules penetrate into the interior cavities of G5 and G6 dendrimers and localize near the core or the boundary (1-H and 3-H) of each generation (Scheme 1)a. Moreover, the lack of cross-peaks between aromatic protons of phenobarbital and the G3 or G4 dendrimers suggests that few drug molecules are entrapped in the cavities of G3 or G4 dendrimers (Fig. 1c and d). Molecular simulations of PAMAM dendrimers' structure showed that lower generation dendrimers ($G < 4$) have an open structure, but that higher generation dendrimers ($G \geq 4$) possess a densely packed surface [33]. Hence, higher generation PAMAM dendrimers ($G \geq 4$) with solvent-filled interior hollows connected by channels along the entire length of the dendrimer are more capable of encapsulating guest molecules. Although the minimization of spin–lattice relaxation times (T_1) of carbons of salicyclic acid (SA) in the presence of G3 and G4 PAMAM dendrimers was achieved in an early study, it does not mean G3 or G4 dendrimers are capable of encapsulating SA because SA could also be aggregated and congested at the surface of dendrimers which is also a factor of reducing the T_1 values of the guest [33]. The cross-peaks between protons of phenobarbital and those of dendrimer in NOESY spectra definitely confirm that the higher generation of PAMAM dendrimers, the higher capability of encapsulating phenobarbital molecules in their interior.

The aqueous solubilities of phenobarbital in the presence of 2 mg/ml G3–G6 PAMAM dendrimer are shown in Fig. 2. All the dendrimers significantly increase the solubility of phenobarbital in water. Surprisingly, lower generation dendrimers increase the solubility of phenobarbital to a higher level than higher ones at the same mass concentration (similar numbers of primary, ternary amine groups, and repeated units for different generations of dendrimers at a fixed mass concentration of 2 mg/ml). Previous studies suggested that the enhanced solubility of hydrophobic drugs was due to hydrophobic interaction, hydrogen-bond formation and electrostatic interaction between guests and dendrimers [34–36]. The hydrophobic interaction and hydrogen-bond formation together contributed to the encapsulation of guests in the cavities of hosts [8]. The



Scheme 1. (a) Proposed localizations of phenobarbital molecules in the cavities of PAMAM dendrimers. The arrows indicate the most possible sites for the PAMAM dendrimers to entrap phenobarbital molecules. (b) Equilibrium of non-charged and negatively charged phenobarbital structures in solutions.

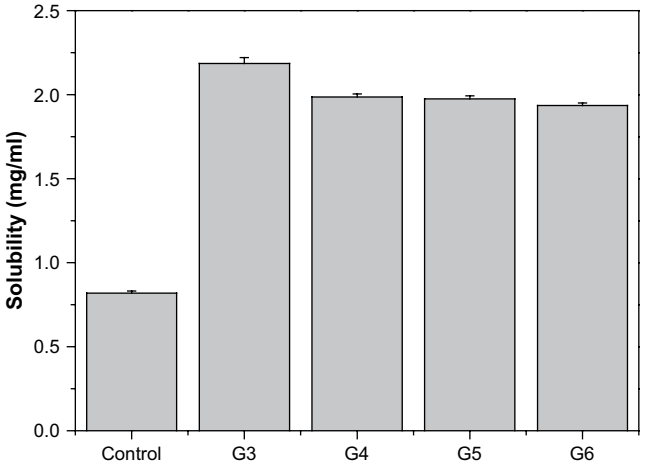


Fig. 2. Aqueous solubilities of phenobarbital in the presence of 2 mg/ml G3, G4, G5, and G6 amine-terminated PAMAM dendrimers.

phenobarbital molecule has different forms in equilibrium in basic solutions: non-charged forms and a negatively charged form (Scheme 1b). The negatively charged form of phenobarbital can be attached to the surface of positively charged PAMAM dendrimers by electrostatic interaction. Thus, we can classify the contribution factors of the solubility enhancement into two parts: the encapsulation and electrostatic interaction. Since higher generation dendrimers are more capable of encapsulating phenobarbital molecules than lower generation dendrimers and the enhanced solubility of phenobarbital decreases with an increase in dendrimer generation (2 mg/ml), we can conclude that lower generation dendrimers are much easier for electrostatic attachment of negatively charged phenobarbital molecules than higher generations at a fixed mass concentration. The decreased electrostatic attachment effect of higher generation dendrimers with respect to the lower ones should not be attributed to the different pH conditions or numbers of primary amine groups at different dendrimer generations, since these are nearly the same at the

Table 1
The characteristics of G3, G4, G5, and G6 amine-terminated PAMAM dendrimers

Generation	Molecular formula	Molecular weight	Number of terminal amino groups	Radius (Å)	Surface amine density (per Å^2)
G3	$C_{302}H_{608}O_{60}N_{122}$	6909	32	18	7.86 E−3
G4	$C_{622}H_{1248}O_{124}N_{250}$	14,215	64	22.5	10.07 E−3
G5	$C_{1262}H_{2528}O_{252}N_{506}$	28,826	128	27	13.98 E−3
G6	$C_{2542}H_{5088}O_{508}N_{1018}$	58,048	256	33.5	18.16 E−3

dendrimer concentration of 2 mg/ml. The reason for the decrease in the electrostatic attachment of phenobarbital seems to be the much more congested primary amine groups on the surface of higher generation dendrimers (Table 1), which causes steric hindrance for the electrostatic attachment in the case of higher generation dendrimers.

In conclusion, 2D-NOESY has proved to be a useful tool to investigate the encapsulation of hydrophobic guests into dendrimer hosts in this communication. Results from 2D-NOESY and aqueous solubility studies clearly demonstrated that higher generation dendrimers are more capable of encapsulating phenobarbital molecules into the interior cavities than lower generations, and that lower generation dendrimers are much easier for the electrostatic attachment of phenobarbital molecules than higher ones at a fixed mass concentration. This conclusion is helpful for the design of new drug delivery systems in the treatment of many diseases. Of course, the systemic pH value, ionic strength, charge and molecular weight of the guest, as well as stability and release profile of the dendrimer–drug complexes under physiological conditions are crucial for the dendrimer-based drug delivery systems. For example, the pH of transition of drug in Scheme 1b and the p*K*s of the primary and ternary amines in dendrimers between different binding states are key factors for designing pH-sensitive controlled release systems. These points will be deeply addressed in the future to further evaluate the potential biological relevance of this promising delivery system and the NOESY NMR technique will play an important role in establishing such delivery devices.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.05.031.

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