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ORIGINAL ARTICLE

Synthesis of conjugates of β-cyclodextrin with polyamidoamine dendrimers and their molecular inclusion interaction with levofloxacin lactate

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Abstract Polyamidoamine (PAMAM) dendrimers of different generations (G2 and G4) conjugated with β-cyclodextrin (β-CD), PAMAM (G2, G4)-CD, were synthesized using substitution reaction from mono-6-iodine-β-cyclodextrin and PAMAM dendrimers. The resulting molecular structures were characterized by NMR, IR. The molecular interaction between various dendrimers and levofloxacin lactate (LFL) were investigated by monitoring the fluorescence of LFL in the presence of dendrimers in buffer solution (pH 7.4) at 25 °C. It was found that the PAMAM (G2, G4)-CD possesses higher sensitizing ability than that of the corresponding parent dendrimers and natural β-CD, and increases concomitantly with the increases of generation and content of β-CD, suggesting that the PAMAM (G2, G4)-CD possesses stronger inclusion ability with LFL. The possible interaction mechanism between PAMAM-CD and LFL was proposed by ^{1}H NMR analysis and theoretical calculation. The results show that the LFL molecule is located at the amine end of dendrimer molecule and along the side of cyclodextrin cavities to form supramolecular complexes. Furthermore, results indicate that the main driving force of the complex could be attributed to the electrostatic interactions and hydrogen bonding between LFL and PAMAM-CD, as well as the synergistic effect of intermolecular forces.

Keywords Polyamidoamine (PAMAM) dendrimer · β-Cyclodextrin · Levofloxacin lactate · Molecular inclusion interaction

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Abbreviation

β-CD	β-Cyclodextrin
PAMAM	Polyamidoamine
LFL	Levofloxacin lactate
PAMAM-CD	Conjugates of β-cyclodextrin with polyamidoamine dendrimers

Introduction

Among many polymer materials, dendrimers have attracted attention in many fields due to their unique structures accompanied by specific properties [1–4]. Polyamidoamine (PAMAM) dendrimers as hyperbranched polymers possess well-defined structures, which allow precise control of size, shape, and terminal group functionality. The PAMAM dendrimer is stable and highly biocompatible and can complex with small molecule drugs at the periphery or interior; its terminal groups can also be conjugated with the antibody genes and other biological active substances. The PAMAM dendrimer has a high efficiency transferring biological active agents [5–7], suggesting potential pharmaceutical applications such as a drug delivery vessel.

Cyclodextrin (CD) and its derivatives have been widely used in biomedical, pharmaceutical, environmental engineering and many other fields. Cyclodextrin polymers not only maintain the characteristics of the parent molecules, but also show unique polymer effects, range of applications in separation analysis technology, biomedicine engineering and other fields [8–10]. Kihara [7] has demonstrated that starburst PAMAM dendrimer (generation 2 or 3) conjugated with α-CD (α-CDE conjugates) in a 1:1 molar ratio can be utilized as a novel nonviral vector for gene and siRNA delivery in vitro and in vivo. A large number of

studies have shown that incorporation of CDs into PAMAM dendrimers provides a single polymer structure from the combination of two types of molecular cavities. This polymer is not only interesting for research in structure–property relationships, but also may be used for important applications.

In recent years, our research group has focused on building multi-functional structures of the supramolecular host–guest cyclodextrin polymers and studying their controlled drug release rates [11–14]; however, more research was needed to understand the effect of the composition of cyclodextrin polymers on their ability to act as a drug carrier.

In the present study, the dendrimers (G2, G4) conjugated with the β-CD were synthesized; products containing different β-CD content could be obtained by controlling the reaction time (Scheme 1). The interaction of these conjugates with LFL was also determined by monitoring the fluoresce spectra of LFL in aqueous solution.

Experiment section

Materials and instrument analyses

Beta-CD was obtained from Shan-Tou Chemical Factory in China, and recrystallized from water twice before using. All other reagents were of analytical grade made in China, and were used as received without further purification.

NMRs measurements were conducted on a Varian Inova 400 spectrometer (Massachusetts) at room temperature with D_2O as a solvent. FT-IR spectroscopy experiments were preformed on a Prestige-21 model fourier transform infrared spectroscopy (Shimadzu, Japan). Fluorescence spectra were recorded on a RF-5301PC fluorescence spectrophotometer (Shimadzu, Japan). Dialysis experiments were using a dialysis membrane with a MWCO of <6000 (Daltons).

Experimental

Synthesis of PAMAM-CD

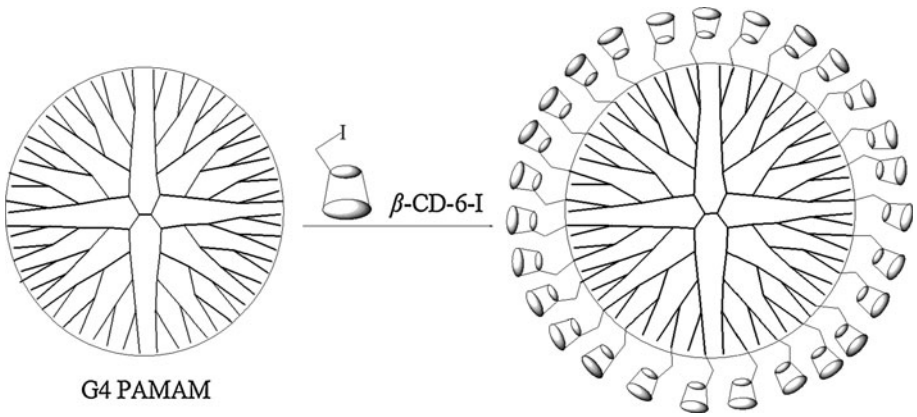
G4 PAMAM 0.25 g (1.76×10^{-5} mol) and 1.7 g (1.37×10^{-3} mol) β-CD-6-I dissolved in DMF, and stirred under nitrogen at 75 °C. The mixture was then dialyzed at room temperature for five days, exchanging the solvent twice a day. PAMAM (G4)-CD was obtained after lyophilization. A similar approach was used to PAMAM (G2)-CD. The average molar ratios of β-CD and PAMAM in synthesized conjugates were controlled by reaction time as shown in Table 1. ^{1}H NMR (D_2O), PAMAM (G2, G4)-CD δ: 4.97 (H1, β-CD), 3.95–3.62 (H3, H5, H6, β-CD), 3.62–3.38 (H2, H4, β-CD), 3.38–3.09 (-CH_2-, PAMAM), 2.99–2.60 (-CH_2-, PAMAM), 2.60–2.48 (-CH_2-, PAMAM), 2.48–2.20 (-CH_2-, PAMAM); IR (KBr tablet), PAMAM (G2, G4)-CD ν/cm^{-1}, 3400, 2935, 1651, 1554, 1437, 1035. Compound PAMAM and β-CD-6-I were prepared according to the reported method [15, 16].

^{1}H NMR studies revealed that, on average, PAMAM (G4) dendrimer (Fig. 7) was successfully conjugated with β-CD molecules. The successful conjugation of PAMAM(G4)-CD dendrimer is indicated by the additional peaks at the chemical shift δ4.97–3.38, which confirmed the attachment of β-CD to PAMAM (G4) dendrimer, and the number amount of β-CD was calculated by comparing

Table 1 Reaction time and average β-CD/PAMAM molar ratios

Sample	Reaction time (h)	β-CD/PAMAM(G2, G4) (mol/mol)[a]
PAMAM(G2)-CD	20	6.2:1
PAMAM(G4)-CD-I	15	11.9:1
PAMAM(G4)-CD-II	20	19.7:1
PAMAM(G4)-CD-III	25	26.4:1

[a] The average molar ratios of β-CD and PAMAM were calculated from ^{1}H NMR spectra of PAMAM (G2, G4)-CD



Scheme 1 Synthesis route to β-cyclodextrin-conjugated PAMAM dendrimer

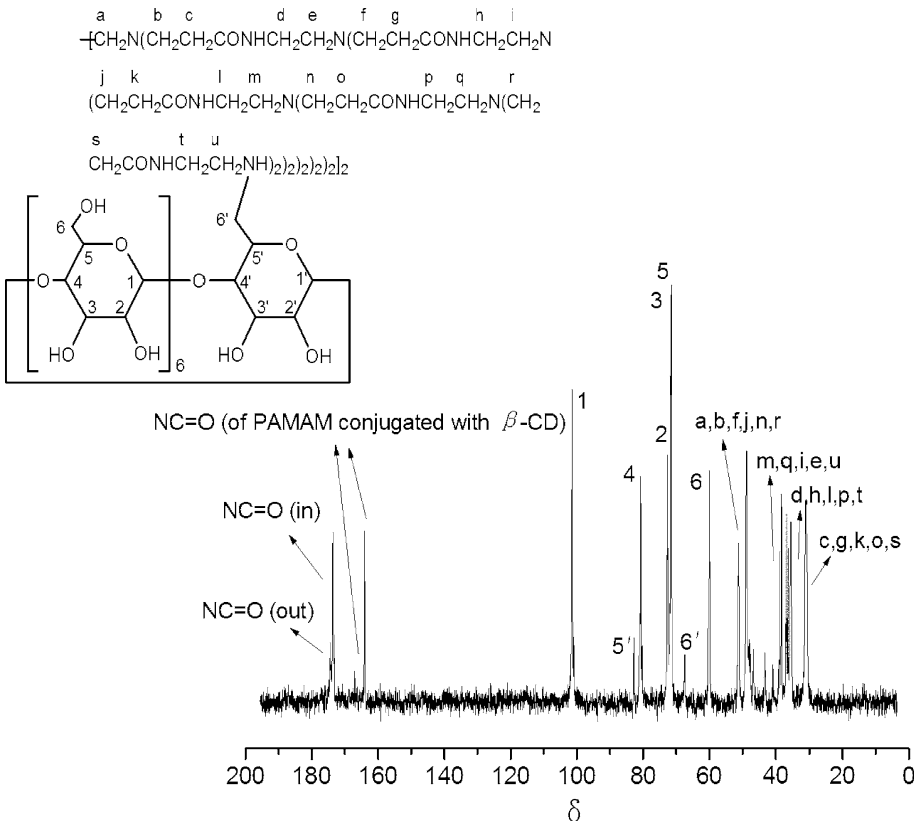


Fig. 1 ^{13}C NMR spectrum of PAMAM (G4)-CD in D_2O

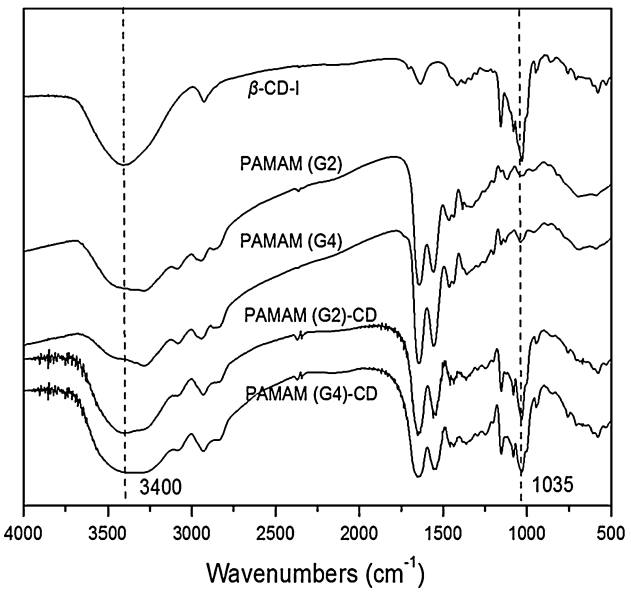


Fig. 2 The FT-IR spectra of β-CD-I, PAMAM (G2, G4) and PAMAM (G2, G4)-CD

the peak integrations of the chemical shift of β-CD at δ4.97 and of PAMAM dendrimer at δ2.34. In addition, the signals from the four CH_2 protons in the interior of the dendrimer shift downfield as compared with parent PAMAM [17], at δ2.34, δ2.54, δ2.73 and δ3.26. This can be attributed conjugation of the PAMAM CH_2 protons with β-CD molecules. Overlapping signals makes it difficult to identify these protons; however, ^{13}C NMR measurements further confirm that PAMAM (G4) dendrimer (Fig. 1) was successfully conjugated with β-CD molecules. There is an upfield chemical shift of NC=O (in and out) which in PAMAM are conjugated with β-CD as compared with the original NC=O (in and out).

The FT-IR spectra of β-CD-I, PAMAM (G2, G4) and PAMAM (G2, G4)-CD are shown in Fig. 2. The main characteristic absorption peaks of two structural units were 1650, 1546 cm^{-1} for C=O and C–N stretching vibration absorption of PAMAM, 1035 cm^{-1} for C–O stretching vibration absorption of CD. A strong broad band found at 3440 cm^{-1} was observed for all samples, which can be assigned as O–H and N–H stretching vibration in β-CD and in PAMAM.

Fluorescence of levofloxacin lactate (LFL) in the presence of conjugates PAMAM-CD

LFL was dissolved in the pH 7.4 phosphate buffer solution, at a concentration of 5.02×10^{-6} M. The fluorescence spectra were measured at room temperature, the excitation wavelength of 310 nm, emission wavelength of 400–550 nm, and slit width of 10 and 5 nm. The fluorescence of LFL were measured in the presence of β-CD and

various dendrimers (concentrations from 0.04 to 1.5×10^{-4}M) under the same conditions as described above.

Results and discussion

The fluorescence spectra of LFL in the presence of various PAMAM-CD conjugates

LFL is the third-generation fluoroquinolone antibiotic drug that inhibits bacterial DNA replication. LFL has been widely used due to a broad antibacterial range and lower toxicity. Its molecular structure is shown in Fig. 3.

Figure 4 shows the fluorescence spectra of the LFL in the presence of different concentrations of various dendrimers PAMAM (G2, G4), PAMAM (G2)-CD and PAMAM (G4)-CD-I-III. Fluorescence intensity of LFL strengthens with both increasing dendrimer generation and increasing molar ratio of β-CD in the conjugates. Fluorescence of LFL was also measured with varying concentrations of conjugate PAMAM-CD. As shown in Fig. 5,

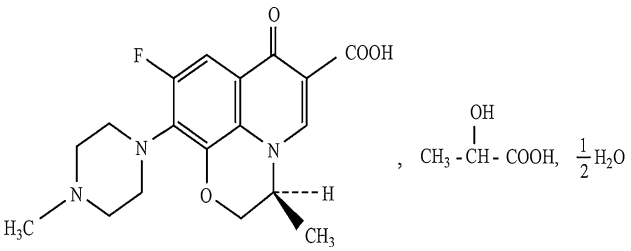


Fig. 3 Molecular structure of levofloxacin lactate

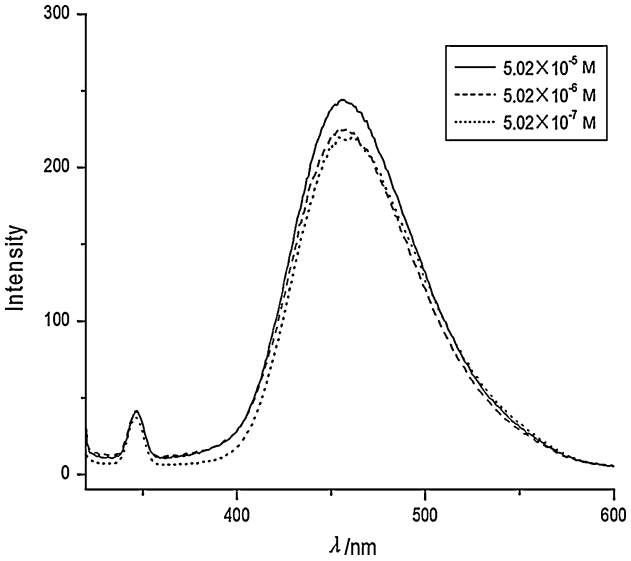


Fig. 5 The effect of LFL's concentration on the fluorescence intensity of LFL

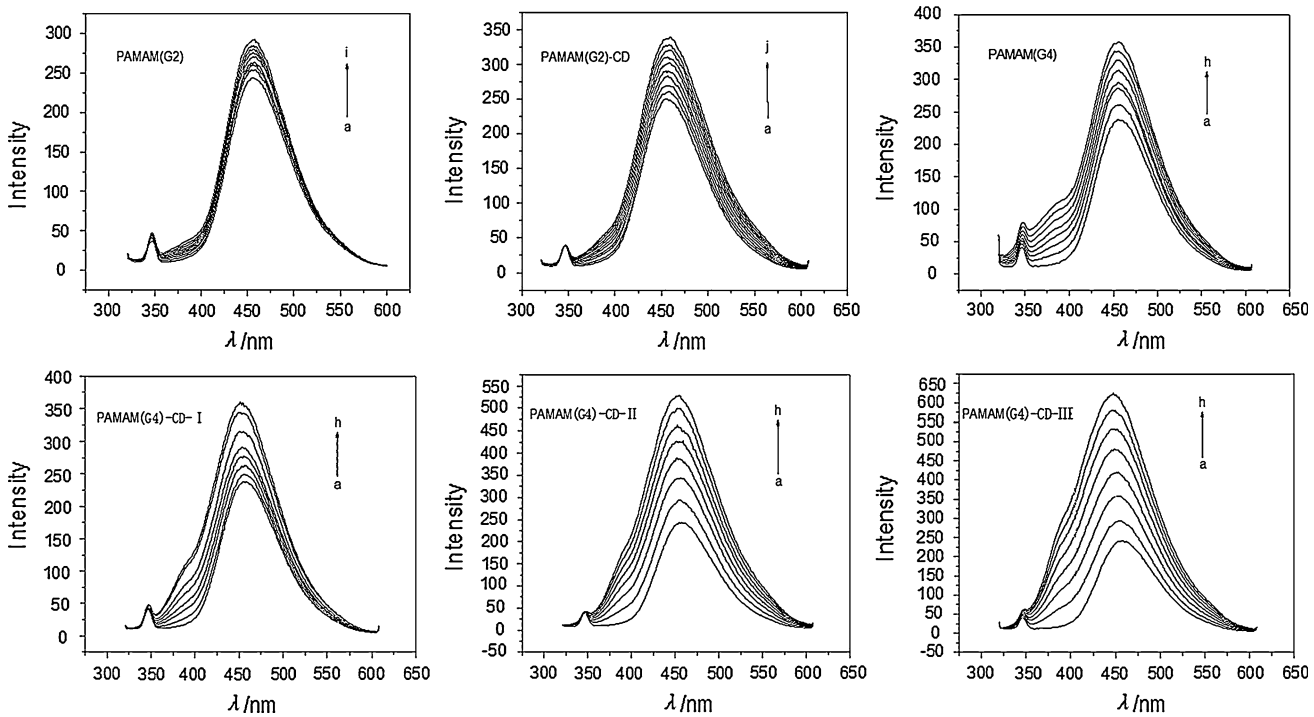


Fig. 4 The effect of PAMAM(G2, G4) and PAMAM(G2, G4)-CD on fluorescence spectra of LFL in phosphate buffer solution (pH 7.4). λex/λem=310 nm/455 nm; CLFL=5.02×10^{-6} M; $C_{Dendrimers}$ from 0 to 1.5×10^{-4}M

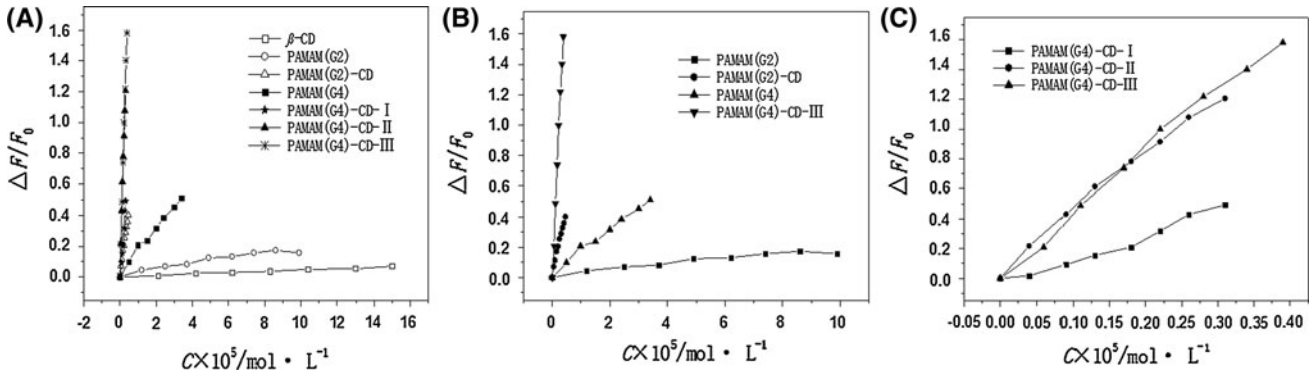


Fig. 6 $\Delta F/F_0$-$C_{\text{Dendrimer}}$ profiles

changes in the intensity of LFL fluorescence were observed at different concentrations, but the degree of change was considerably smaller.

The synergistic interaction of PAMAM-CD and LFL

Figure 4 shows that dendrimers can increase the fluorescent intensity of LFL while Fig. 6 shows conjugates containing dendrimers of different generations and contents of β-CD affect the fluorescence of LFL to different extents. At a host compound concentration of 3×10^{-6} M, samples from greatest to least effect were PAMAM (G4)-CD-III (1.49)>PAMAM (G4)-CD-II (1.17)>PAMAM (G4)-CD-I (0.48)>PAMAM (G2)-CD (0.32)> PAMAM (G4) (0.06)> PAMAM (G2) (0.015)> β-CD (0.007), in which PAMAM (G4)-CD-III is 4.67-fold greater than PAMAM (G2)-CD, while PAMAM (G4)-CD-III is 24.8-fold and 212.9-fold that of PAMAM (G4) and β-CD, respectively. That is, the sensitizing rate of dendrimers conjugated with β-cyclodextrin is larger than non-conjugated dendrimers or natural cyclodextrin, and fluorescence is greatest in conjugates containing higher generation dendrimers and greater amounts of cyclodextrin.

The effect of PAMAM dendrimers on LFL fluorescence suggests that in the pH 7.4 buffer solution, the interaction of LFL molecule –COO– with the protonated primary amine of PAMAM lowers the degree of freedom of molecular movement, preventing collision deactivation and

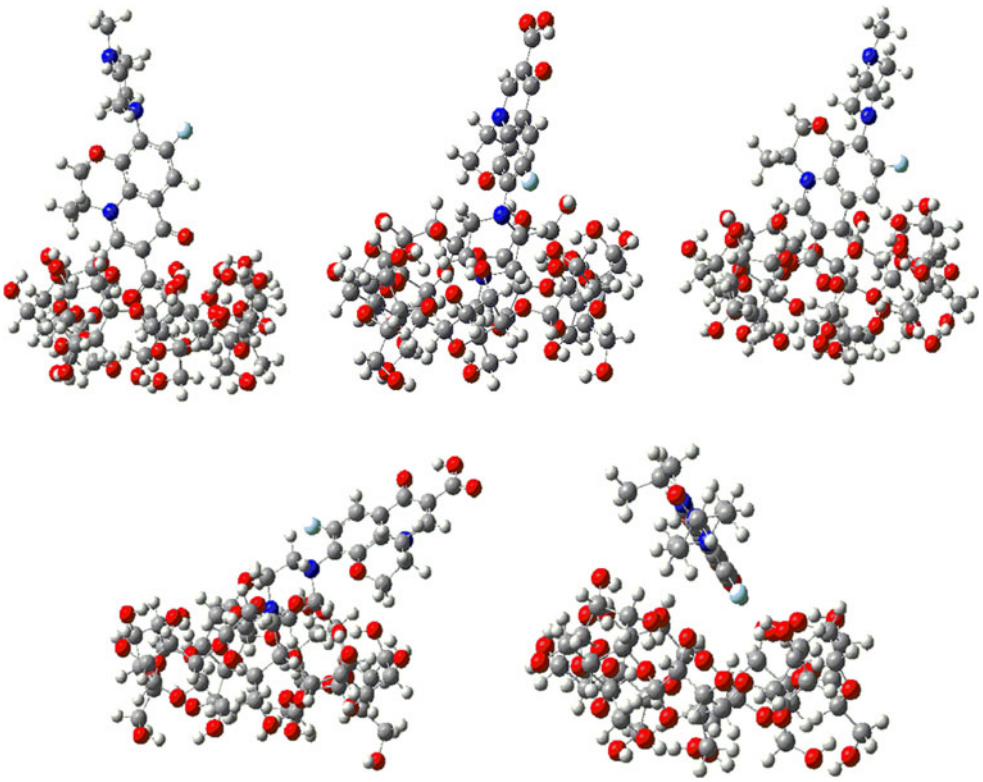


Fig. 7 The optimized structures of β-cyclodextrin/levofloxacin lactate complex

therefore increasing quantum yield, increasing fluorescence intensity accordingly. These results are consistent with the interactions between PAMAM dendrimers and bovine serum albumin reported by Pałecz B. [18]. In addition, the cyclodextrin-conjugated dendrimers affect fluorescence to a greater extent then either material alone, indicating that there are the synergistic intermolecular forces in these supramolecular complexes.

In order to understand the interaction mechanism of the cyclodextrin-conjugated dendrimers with the LFL, the molecular interaction behavior of the LFL and the natural cyclodextrin has been investigated by spectroscopy and theoretical calculation. Experiments show that although the β-CD also has an effect on LFL, it's so weak that we measured the binding constant using fluorescence titration, indicating the existence of weak intermolecular forces. In order to confirm this result, the theoretical interactions between β-CD and LFL have been investigated. The total energy E and interaction energy ΔE, $\Delta E'$ have been calculated using Gaussian 03 calculation software. As the system is too large, and the computer configuration is limited, the quantitative calculation was completed using the semi-empirical PM3. Five minima were obtained for the ground state for β-CD/LFL complex as well as the structure and energy, as indicated by Fig. 7 and Table 2, respectively. E is the total energy (atomic units), ZPE is zero point energy, ΔE is the interaction energy, and $\Delta E'$ is the interaction energy corrected by zero-point energy. The structural parameters indicate that this interaction is weak intermolecular forces, which is consistent with the experimental results.

Table 2 Calculated total energies E (in a.u.), zero-point energies (ZPE, in kJ/mol), interaction energy ΔE, $\Delta E'$ (in kJ/mol) for the ground state of β-CD/LFL complex

Species	E	ZPE	ΔZPE	ΔE	$\Delta E'$
LFL	−0.26268173	959.7844	–	–	–
β-CD	−2.28646782	3128.1087	–	–	–
1	−2.58192229	4069.8316	18.1	−86.0	−67.9
2	−2.58964664	4070.5238	17.4	−106.0	−88.6
3	−2.58736943	4077.8227	10.1	−100.3	−90.2
4	−2.59544624	4078.7578	9.1	−121.5	−112.4
5	−2.58833280	4070.7139	17.2	−102.8	−85.6

NMR spectroscopy is a very useful technique to investigate the intermolecular interactions in solutions [17, 19]. In order to further understand the synergistic effect of molecular interaction, we measured the ^{1}H NMR spectra of conjugates PAMAM-CD in D_2O in the presence of LFL (Fig. 8). From the NMR spectrum of the mixed system PAMAM-CD with the LFL (PAMAM-CD + LFL), the chemical shifts of two segments PAMAM and cyclodextrin can be clearly observed. The chemical shift of CH_2 protons in PAMAM segment, the H3, H5 protons in the cavity of β-CD and Hc of proton lactic acid segment are essentially unchanged, while the chemical shifts of H2 and H4 in the cavity of β-CD occurred to a greater degree ($\Delta\delta = 0.19$). So, it is inferred that LFL did not enter into the interior of PAMAM or β-CD moiety of molecular chains, but sits in between the amino group and side of cyclodextrin molecules to form supramolecule as shown in Fig. 9. In addition, the chemical shift of LFL H5, H8, Hd protons in this system also occurred to varying degrees. Due to the carboxyl anion, the chemical shift of H5 and Hd protons shifted upfield ($\Delta\delta$ 0.12 and $\Delta\delta$ 0.20), while the H8 shift downfield ($\Delta\delta$ 0.18) was attributed to effects of the hydrogen bonds between F atoms and the cyclodextrin

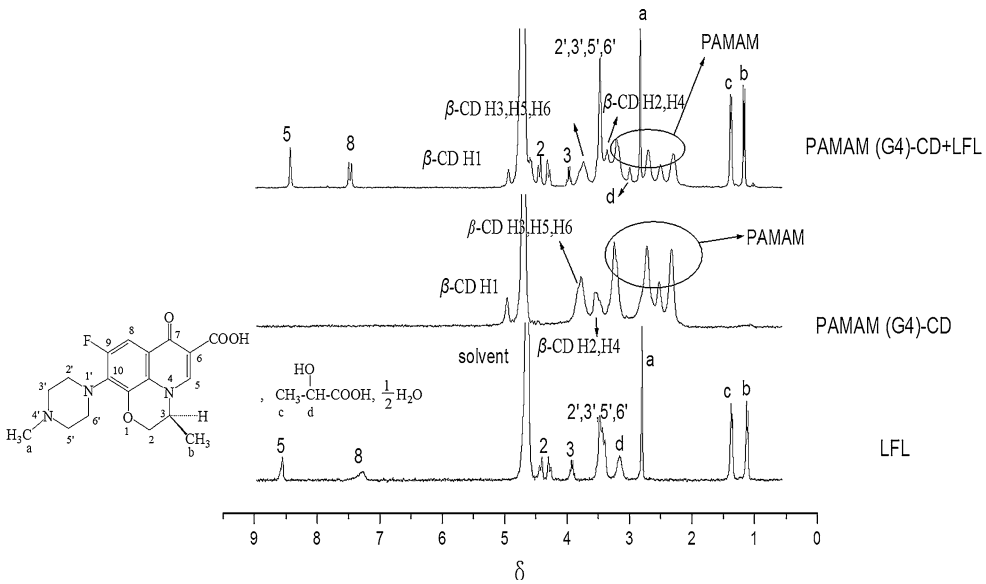


Fig. 8 ^{1}H NMR spectra of LFL, PAMAM(G4)-CD and PAMAM(G4)-CD + LFL in D_2O

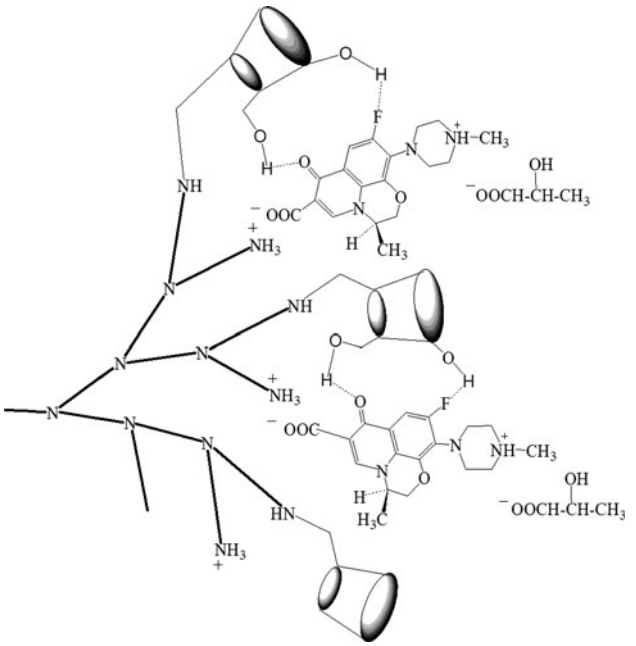


Fig. 9 Possible interaction mechanism between PAMAM-CD and LFL in H_2O

hydroxyl groups. Thus, the main driving force of the system could be attributed to the electrostatic interaction of LFL molecules in the carboxyl anion and protonated amine as well as the hydrogen bond between the LFL and PAMAM-CD.

These results suggest that the increase in LFL fluorescence with cyclodextrin-conjugated PAMAM dendrimers is from a synergistic effect of molecular interactions between dendrimers PAMAM-CD as a host and guest, rather than the simple sum of two segments PAMAM and cyclodextrin. The results of these experiments should help our understanding of the host–guest supramolecule behavior, and provide some guidance for the design of the structure of host–guest supramolecule system in the functional materials with a variety of functions.

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References

1. Tomita, S., Sato, K., Anzai, J.I.: Preparation of dendrimer-loaded microcapsules by a layer-by-layer deposition of polyelectrolytes. Mater Sci. Eng. **6**, 2024–2028 (2009)
2. Auten, B.J., Lang, H.F., Chandler, B.D.: Dendrimer templates for heterogeneous catalysts: bimetallic Pt-Au nanoparticles on oxide supports. Appl Catal B **81**, 225–235 (2008)
3. Casas, A., Battah, S., Venosa, G.D., Dobbin, P., Rodriguez, L., Fukuda, H., et al.: Sustained and efficient porphyrin generation in vivo using dendrimer conjugates of 5-ALA for photodynamic therapy. J. Controlled Release **135**, 136–143 (2009)
4. Yang, W.J., Cheng, Y.Y., Xu, T.W., Wang, X.Y., Wen, L.P.: Targeting cancer cells with biotin–dendrimer conjugates. Eur. J. Med. Chem. **44**, 862–868 (2009)
5. Sweeta, D.M., Kolhatkarb, R.B., Rayd, A., Swaanb, P., Ghandehari, H.: Transepithelial transport of PEGylated anionic poly(amidoamine) dendrimers: implications for oral drug delivery. J. Controlled Release **138**, 78–85 (2009)
6. Najlahl, M., Freemana, S., Attwooda, D., Emanuele, A.D.: In vitro evaluation of dendrimer prodrugs for oral drug delivery. Int. J. Pharm. **336**, 183–190 (2007)
7. Kihara, F., Arima, H., Tsutsumi, T., Hirayama, F., Uekama, K.: In vitro and in vivo gene transfer by an optimized α-cyclodextrin conjugate with polyamidoamine dendrimer. Bioconjugate Chem. **14**, 342–350 (2003)
8. Demir, S., Kahraman, M.V., Bora, N., Apohan, N.K., Ogan, A.: Preparation, characterization, and drug release properties of poly(2-hydroxyethyl methacrylate) hydrogels having β-cyclodextrin functionality. J. Appl. Polym. Sci. **109**, 1360–1368 (2008)
9. Uekama, K., Hirayama, F., Arima, H.: Recent aspect of cyclodextrin-based drug delivery system. J. Incl. Phenom. Macrocycl. Chem. **56**, 3–8 (2006)
10. Xia, Y.Y., Wan, J.M.: Preparation and adsorption of novel cellulosic fibers modified by β-cyclodextrin. Polym. Adv. Technol. **19**, 270–275 (2008)
11. Huang, Y., Fan, X.D.: Synthesis and properties of hydrogels of poly (acrylic-co-acroloyl β-cyclodextrin). J. Appl. Polym. Sci. **113**, 3068–3077 (2009)
12. Huang, Y., Fan, X.D., Zhang, N.N.: Study on immobilization of β-cyclodextrin onto poly(vinyl alcohol) and its controlled drug release behavior. Acta Polym. Sin. **6**, 854–858 (2004)
13. Tian, W., Fan, X.D., Kong, J., Liu, T., Liu, Y.Y., Huang, Y., et al.: Cyclodextrin-based hyperbranched polymers: molecule design, synthesis, and characterization. Macromolecules **42**, 640–651 (2009)
14. Tian, W., Fan, X.D., Liu, Y.Y., Jiang, M., Huang, Y., Kong, J.: β-Cyclodextrin polymer brushes based on hyperbranched polycarbosilane: synthesis and characterization. J. Polym. Sci. Part A Polym. Chem. **46**, 5036–5052 (2008)
15. Santo, M., Fox, M.A.: Hydrogen bonding interactions between star-burst dendrimers and several molecules of biological interest. J. Phys. Org. Chem. **12**, 293–307 (1999)
16. Bonomo, R.P., Cucinotta, V.D., Alessandro, F., Impellizzeri, G., Maccarrone, G., Vecchio, G., et al.: Conformatinal features and coordination properties of functionalized cyclodextrins. Formation, stability, and structure of proton and copper (II) complexes of Histamine-Bearing β-cyclodextrin in aqueous solution. Inorg. Chem. **30**, 2708–2713 (1991)
17. Hu, J.J., Cheng, Y.Y., Ma, Y.R., Wu, Q.L., Xu, T.W.: Host-guest chemistry and physicochemical properties of the dendrimer-mycophenolic acid complex. J. Phys. Chem. B **113**, 64–74 (2009)
18. Pałecz, B., Klajnert, B., Cawska, L.S., Bryszewska, M.: Interactions between PAMAM dendrimers and bovine serum albumin. Biochim. Biophys. Acta **1648**, 115–126 (2003)
19. Setijadi, E., Tao, L., Liu, J.Q., Jia, Z.F., Boye, C., Davis, T.P.: Biodegradable star polymers functionalized with-cyclodextrin inclusion complexes. Biomacromolecules **10**, 2699–2707 (2009)