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Design of interior-functionalized fully acetylated dendrimers for anticancer drug delivery

Jingjing Hu[b], Yunzhang Su[a], Hongfeng Zhang[a], Tongwen Xu[b,*], Yiyun Cheng[a,*]

[a] School of Life Sciences, East China Normal University, Shanghai 200241, PR China
[b] Department of Chemistry, University of Science and Technology of China, Hefei 230026, PR China

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ABSTRACT

In this study, dendrimers was synthesized by introducing functional groups into the interior pockets of fully acetylated dendrimers. NMR techniques including COSY and 2D-NOESY revealed the molecular structures of the synthesized dendrimers and the encapsulation of guest molecule such as methotrexate within their interior pockets. The synthesized polymeric nanocarriers showed much lower cytotoxicity on two cell lines than cationic dendrimers, and exhibited better performance than fully acetylated dendrimers in the sustained release of methotrexate. The results provided a new strategy in the design of non-toxic dendrimers with high performance in the delivery of anti-cancer drugs for clinical applications.

1. Introduction

Dendrimers are new class of artificial macromolecules with tree-like topological structure, nano-scale size, excellent monodisperisty, and well-defined numbers of interior pocket and surface functionality [1–4]. Due to the unique characteristics of dendrimers, they have been used as light- and energy-harvesting materials, catalysts, templates for nanomaterial synthesis, and especially as devices for pharmaceutical and biomedical applications during the past decade [3,5–23]. Drugs or genes can be either encapsulated within the interior pockets of dendrimers through non-covalent strategies such as hydrophobic, ionic, and hydrogen-bond interactions, or conjugated to the surface functionalities of dendrimers via covalent methods [5,24,25].

Among the numerous dendrimers used for drug delivery, poly(amidoamine) (PAMAM) and poly(propylene imine) (PPI) dendrimers are the two commercially available and most investigated ones [7]. They were reported to effectively improve solubility, stability, and deliver efficacy, decrease side-effects, and tailor pharmacokinetic and pharmacodynamic behaviors of several families of drugs [14], which reveal the promising future of dendrimer-based drug delivery systems. However, the approval of a drug formulation by Food and Drug Administration (FDA) and its clinical applications depend on both therapeutic/diagnostic efficacy and safety [5]. Cationic PAMAM and PPI dendrimers showed excellent drug delivery efficacy but exhibited high cytotoxicity on numerous cell lines and serious hemolytic activity on red blood cells [26,27]. Cationic groups on dendrimer surface are the predominant factor in generating cytotoxicity and hemolytic activity [26,27]. These cationic groups interact with the phosphates on the cell membrane, which leads to disturbance of lipid bilayers and leakage of intracellular components [28]. Furthermore, cationic dendrimers have a rapid rate of blood clearance, resulting in limited bioavailability of the administrated dendrimer/drug formulation [29].

Acetylation, PEGylation, and glycosylation are effective strategies for removing cationic charges on dendrimer surface and reducing cytotoxicity and hemolytic activity of cationic dendrimers [5,30–32]. Among them, acetylation is a facile, highly efficient method, and thus the most favorable one [33]. The degree of acetylation on dendrimer surface can be easily tailored by the addition of proper amounts of acetic anhydride [8,34,35]. The acetylated dendrimer maintains the high penetration ability and significantly reduces the cytotoxicity of cationic dendrimer [32]. However, acetylated dendrimers have serious limitations when they were used as drug vehicles. Surface charge on cationic dendrimer plays an important role in drug loading and delivery especially in the enhancement of aqueous solubility of hydrophobic drugs [36,37]. Acetylated dendrimers failed to load these

* Corresponding authors.
E-mail addresses: twxu@ustc.edu.cn (T. Xu), yycheng@mail.ustc.edu.cn (Y. Cheng).

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hydrophobic drugs due to the neutralization of surface amines on cationic dendrimers [36]. Though acetylated dendrimers are capable of encapsulating ionic drugs within their interior pockets at low pH mediums when the tertiary amine groups of PAMAM or PPI dendrimer are quaternized [38], the charge property of acetylated dendrimer interior is pH-dependent and the quaternized amines in the interior will be deprotonated and non-charged at physiological conditions, leading to a burst release of encapsulated drugs after its administration [38].

To decrease the cytotoxicity and maintain the high drug loading/sustained release abilities of cationic dendrimers, we proposed a new class of non-toxic dendrimers with high performance for anticancer drug delivery by introducing functional groups into the interior pockets of fully acetylated dendrimers. Previous studies have demonstrated that PAMAM and PPI dendrimers have large numbers of non-polar pockets, and these pockets are capable of loading guest molecules with molecular weights up to 800 Da [39]. In addition, PAMAM and PPI dendrimers have similar numbers of interior tertiary amines and surface primary amines ($2^{n+2}-2$ versus 2^{n+2} for PAMAM and $2^{n+1}-2$ versus 2^{n+1} for PPI, respectively, where n is dendrimer generation). These interior tertiary amines can react with a number of chemicals such as methyl iodide, ethylene oxide, and 1,3-propane sultone (PS) through quaternization [40–42], which allows the modification of dendrimer interior pockets with different functional groups. The introduced quaternary ammonium ions and other functional groups such as hydroxyl and sulfonate groups in the dendrimer interior are responsive for encapsulation/conjugation of various guests via non-covalent or covalent strategies. These quaternary ammonium ions are stable at physiological condition, which avoids the pH-dependent protonation/deprotonation behaviors of fully acetylated dendrimers [39].

In the present study, fully acetylated generation 4 (G4) and G5 PAMAM and G4 PPI dendrimers were synthesized. The interior pockets of acetylated PAMAM and PPI dendrimers were functionalized with 1,3-propane sultone which generates zwitterions in the dendrimer pockets (Scheme 1). The complex structures of the synthesized dendrimers with an anticancer drug (methotrexate sodium) were characterized, evaluated for sustained drug release, and investigated for cytotoxicity and drug efficacies in two cancer cell lines.

2. Materials and methods

2.1. Materials

G4 and G5 ethylenediamine (EDA)-cored and primary amine-terminated PAMAM dendrimers with 64 and 128 surface functionalities respectively were purchased from Dendritech Inc. (Midland, MI). G4 diaminobutane (DAB)-cored and primary amine-terminated PPI dendrimer with 32 surface functionalities was purchased from Sigma–Aldrich Inc. (St. Louis, MO). 1,3-propane sultone was purchased from Aladdin Inc. (Shanghai, China). *N,N*-Dimethyl formamide (DMF), acetic anhydride, triethylamine, and ethyl ether were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methotrexate sodium was purchased from Jingkang Pharmaceutical Technology Co, Ltd (Jiangsu, China). Acridine orange (AO) and ethidium bromide (EB) were gifts from the school of Life Science, East China Normal University.

2.2. Synthesis of surface-acetylated PAMAM and PPI dendrimers (Ac-G4, Ac-G5 PAMAM, and Ac-G4 PPI)

Acetylation of PAMAM and PPI dendrimers were performed according to a well-established method (Step 1 in Scheme 1). PAMAM (G4 PAMAM 40.00 mg, 2.81 μmol or G5 PAMAM 40.00 mg, 1.39 μmol) or PPI (G4 PPI 41.70 mg, 11.87 μmol) dendrimer was dissolved in 5 mL methanol, followed by the addition of excess amounts of acetic anhydride (5 equivalents per dendrimer surface primary amine to ensure completely acetylation) and triethylamine (1.2 equivalents per equivalent of acetic anhydride to neutralize the acetic acid during acetylation). The mixture was stirred at room temperature for 24 h. The excess acetic anhydride and side products in the mixtures were removed by extensive dialysis (molecular weight cut off of 3500 Da for Ac-G4 and Ac-G5 PAMAM dendrimers and 1000 Da for Ac-G4 PPI dendrimer, respectively) against phosphate buffer (0.2 M Na_2HPO_4/NaH_2PO_4, pH = 7.5) and de-ionized water for two days. The purified samples were lyophilized and white powders were obtained and stored at 4 °C before use. The degrees of acetylation of the samples were characterized by ^{1}H NMR spectroscopy.

^{1}H NMR for Ac-G4 PAMAM in D_2O: H_a, 2.288 ppm (−NCH_2CH_2CONH−); H_b, 2.525 ppm (−$CONHCH_2CH_2N$−); H_c, 2.709 ppm (−NCH_2CH_2CONH−); $H_{d,b',d'}$, 3.149 ppm (H_d, −$CONHCH_2CH_2N$−; $H_{b',d'}$, −$CONHCH_2CH_2NHCOCH_3$); H_e, 1.828 ppm (−$NHCOCH_3$).

Ac-G5 PAMAM: H_a, 2.297 ppm (−CH_2CONH−); H_b, 2.529 ppm (−$CONHCH_2CH_2N$−); H_c, 2.714 ppm (−NCH_2CH_2CONH−); $H_{d,b',d'}$, 3.159 ppm (H_d, −$CONHCH_2CH_2N$−; $H_{b',d'}$, −$ONHCH_2CH_2NHCOCH_3$); H_e, 1.835 ppm (−$NHCOCH_3$).

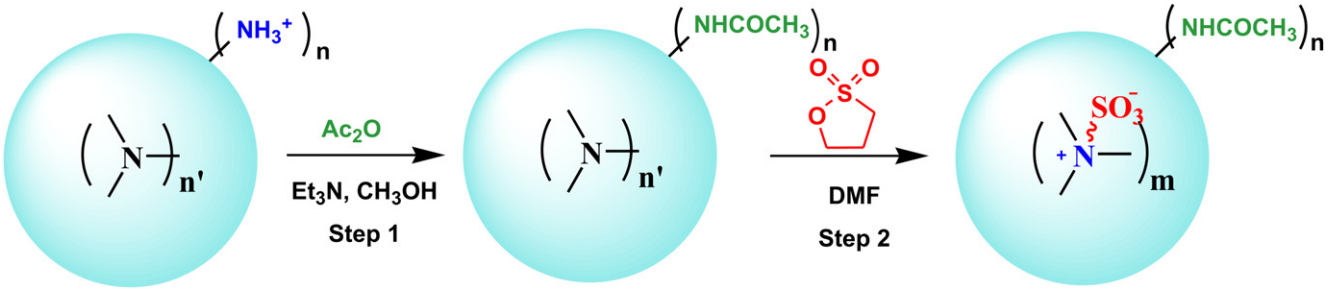
Ac-G4 PPI: $H_{A,A'}$, 1.639 ppm (H_A, −$NCH_2CH_2CH_2N$−; $H_{A'}$, −$NCH_2CH_2CH_2NHCOCH_3$); H_B, 2.541 ppm (−$NCH_2CH_2CH_2N$−), $H_{B'}$, 2.634 ppm (−$NCH_2CH_2CH_2NHCOCH_3$); H_C, 3.071 ppm (−$NCH_2CH_2CH_2NHCOCH_3$), H_D, 1.461 ppm (−$NCH_2CH_2CH_2CH_2N$−); H_E, 1.854 ppm (−$NHCOCH_3$).

2.3. Synthesis of surface-acetylated and interior-functionalized PAMAM and PPI dendrimers (Ac-G4-PS, Ac-G5-PS PAMAM, and Ac-G4-PS PPI)

The Ac-G4 PAMAM, Ac-G5 PAMAM, or Ac-G4 PPI dendrimer was dissolved in 5 mL DMF, followed by the addition of excess amounts of 1,3-propane sultone. A ring opening reaction occurs between 1,3-propane sultone and the tertiary amine groups of PAMAM and PPI dendrimers, yielding a zwitterion consisting of both quaternary ammonium and sulfonate groups (Step 2 in Scheme 1) [43]. The mixture was stirred at room temperature for 24 h, the products were precipitated in ethyl etherand the solvents were removed under reduced pressure, the light yellow solid was re-dissolved in de-ionized water, and dialyzed against de-ionized water (3500 Da for Ac-G4-PS and Ac-G5-PS PAMAM, and 1000 Da for Ac-G4-PS PPI) for two days. The purified samples were lyophilized and light yellow powders were obtained and stored at 4 °C before use. The interior-functionalization degrees of the samples were characterized by ^{1}H NMR spectroscopy.

^{1}H NMR for Ac-G4-PS PAMAM in D_2O: H_a, 2.367 ppm (−NCH_2CH_2CONH−); H_b, 2.634 ppm (−$CONHCH_2CH_2N$−); H_{b+}, 2.695 ppm (−$CONHCH_2CH_2N^+$−); H_c, 2.833 ppm (−NCH_2CH_2CONH−); H_{c+}, 2.824 ppm (−$N^+CH_2CH_2CONH$−); H_d, 3.201 ppm (−$CONHCH_2CH_2N$−); H_{d+}, 3.245 ppm (−$CONHCH_2CH_2N^+$−); $H_{b',d'}$, 3.153 ppm (−$CONHCH_2CH_2NHCOCH_3$); H_e, 1.831 ppm (−$NHCOCH_3$); H_α, 3.498 ppm (−$N^+CH_2CH_2CH_2SO_3^-$); H_β, 1.867 ppm (−$N^+CH_2CH_2CH_2SO_3^-$), overlapped with protons H_e of dendrimer; H_γ, 2.824 ppm (−$N^+CH_2CH_2CH_2SO_3^-$), overlapped with the protons H_c of dendrimer.

Ac-G5-PS PAMAM: H_a, 2.486 ppm (−NCH_2CH_2CONH−); H_{a+}, 2.599 ppm (−$N^+CH_2CH_2CONH$−); H_b, 2.811 ppm (−$CONHCH_2CH_2N$−); H_{b+}, 2.914 ppm (−$CONHCH_2CH_2N^+$−); H_c, 2.974 ppm (−NCH_2CH_2CONH−); H_{c+}, 3.072 ppm (−$N^+CH_2CH_2CONH$); H_d, 3.339 ppm (−$CONHCH_2CH_2N$−); H_{d+}, 3.385 ppm (−$CONHCH_2CH_2N^+$−); $H_{b',d'}$, 3.220 ppm (−$CH_2CH_2NHCOCH_3$); H_e, 1.891 ppm (−$NHCOCH_3$); H_α, 3.543 ppm (−$N^+CH_2CH_2CH_2SO_3^-$); H_β, 1.924 ppm



Scheme 1. Synthesis Route of Acetylated and Surface Acetylated and Interior-functionalized Dendrimers (with 1,3-Propane Sultone).