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Original article

Low generational polyamidoamine dendrimers to enhance the solubility of folic acid: A "dendritic effect" investigation

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ABSTRACT

Low generational (G0–G2, G for generation) polyamidoamine (PAMAM) dendrimers were investigated as enhancers to improve the aqueous solubility of folic acid at pH 11 and pH 5. In these two cases, the solubility of folic acid increases with both the dendrimer concentration and generation. However, the solubilization mechanism is different. The electrostatic interaction between the primary amines of dendrimers and the ionized carboxylic groups of folic acid dominates the dissolution process at pH 11, while the increase of the solubility of folic acid at pH 5 is attributed to the hydrophobic encapsulation inside the dendrimer molecules. In addition, for comparison ethylenediamine was used as a small molecule control to examine the "dendritic effect" in the dendrimer-related solubilization process. Interestingly, PAMAM dendrimers exhibit, at pH 5, a significant superiority over ethylenediamine in enhancing solubility, whereas this "dendritic effect" cannot be observed under the basic condition.

1. Introduction

The aqueous solubility of drugs is a vital feature for their bioavailability which governs their dissolution and transfer process and is thus critical for drug delivery and drug development [1]. A number of newly discovered drugs have failed in commercialization due to their poor water solubility and cell membrane permeability [2]. To overcome this issue, various solubility-enhancing techniques are developed, and many enhancing auxiliary techniques are investigated to improve the dissolution of the drugs exhibiting a low aqueous solubility [3]. Among these auxiliary solubilizing enhancers, dendrimers are ideal candidates due to their distinctive and interesting dendritic architecture which make them admirably feasible in versatile biological applications [4,5]. In particular, polyamidoamine (PAMAM) dendrimer is one of the most popular species and is extensively investigated as an enhancer to improve the dissolution of hydrophobic drugs, such as ketoprofen [6], ibuprofen [7], aceclofenan [8], and riboflavin [9]. PAMAM dendrimers present primary amines on the surface which may electrostatically interact with the negatively charged moieties in the drug molecules. Besides, with varying dimensions the internal cavities of the molecules provide a hydrophobic environment that can encapsulate hydrophobic drugs in water. In addition, the tertiary amines of the dendrimeric structure may interact with certain functional groups of the drug molecules *via* hydrogen bond formation. Altogether, these characteristic properties make dendrimers suitable for drug solubilization.

Folic acid (Fig. 1a) is a well known member of the vitamin B family as well as a tumor-targeting molecule thanks to its high affinity toward the folate receptor (FR) [10]. It is essential for a variety of bodily functions, such as promoting rapid cell division and growth during infancy and pregnancy, and acting as coenzyme in the regeneration of methionine from homocysteine [11]. A folic acid deficiency can result in many health problems and symptoms, including neural tube defects, diarrhea, macrocytic anemia, pregnancy complications, and mental confusion, as examples [12]. The solubility of folic acid is thus crucial for its applications, nevertheless it is practically insoluble in water with a solubility reported as 1.6 mg/L at 25 °C [13]. Hence, we have interests in solubility enhancement of folic acid which is rarely reported in the literatures. Considering the costly preparation and purification of high generational molecules, dendrimer with a lower generation is of special interest for us to start the primary study. In this work, G0–G2 PAMAM dendrimers were synthesized and investigated for improving the solubility of folic acid. For the purpose of comparison, ethylenediamine (EDA, Fig. 1a) was chosen as a small-molecule reference. Although it is widely believed that the

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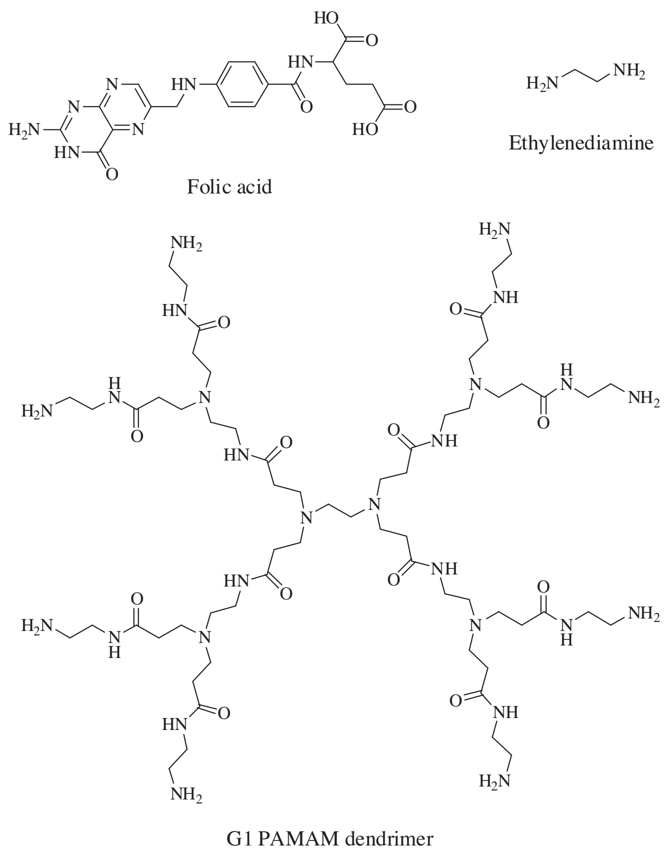

Fig. 1. Molecular structure of folic acid, ethylenediamine and G1 PAMAM dendrimer.

dendritic architecture accounts for the enhancing capability of dendrimers, few publications have made direct comparison between dendrimers and small molecules in terms of solubility performance. In order to reveal the possible "dendritic effect" [14,15] in the dissolution of folic acid, the enhancement of PAMAM dendrimers and EDA on folic acid solubility was evaluated, both at pH 5 and pH 11. In addition, we believe that discovery of the solubilization pattern of the drug molecules under acidic/basic conditions will help us improve drug dissolution and drug delivery.

2. Experimental

2.1. Materials

Folic acid, methyl acrylate and ethylenediamine were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methyl acrylate and ethylenediamine were distilled before use. Double-distilled water was used to prepare all the solutions for solubility studies.

2.2. Synthesis of PAMAM dendrimers

PAMAM dendrimers were synthesized using the classic divergent method developed by Tomalia *et al.* [16]. In brief, ethylenediamine (0.45 g, 7.49 mmol) in MeOH (10 mL) was added to methyl acrylate (5.15 g, 59.8 mmol) and the solution was stirred at 25 °C for 24 h under nitrogen. The solvent and excess methyl acrylate were removed by rotary evaporation and then under vacuum to give an intermediate product bearing four terminal methyl ester groups (2.98 g, 98.6%). Subsequently, ethylenediamine (4.93 g, 82.2 mmol) was added to a methanol solution

Table 1
Characteristic data of the G0/G1/G2 PAMAM dendrimers.

Generation	Molecular formula	Number of primary amine groups	Number of tertiary amine groups
G0	$C_{22}H_{48}O_4N_{10}$	4	2
G1	$C_{62}H_{128}O_{12}N_{26}$	8	6
G2	$C_{142}H_{288}O_{28}N_{58}$	16	14

(10 mL) of that product (1.0 g, 2.47 mmol). The reaction solution was stirred at 25 °C for 24 h under nitrogen, and then the solvent and excess ethylenediamine were removed. The residue was washed with ether and concentrated under vacuum to give a product bearing four terminal amino groups, defined as G0 PAMAM dendrimer (1.23 g, 96.3%). By repeating the Michael addition and amidation reaction mentioned above, G1 (yield: 96.7%, Fig. 1a) and G2 (yield: 97.3%) PAMAM dendrimers were synthesized, and their structures confirmed by ^{1}H NMR and IR spectra (see Supporting information). The characteristics of these PAMAM dendrimers are listed in Table 1.

2.3. Solubility measurements

The solubility of folic acid in the presence of PAMAM dendrimers was determined at pH 5 and pH 11. These two pH values were chosen to provide typical acidic/basic conditions because we believed the ionization status of folic acid significantly affects its solubilization process. The concentration of dendrimers is in the range from 1 mmol/L to 10 mmol/L. Sodium hydroxide and hydrochloric acid were used to adjust the pH value of the sample solutions. The sample preparation is similar for each system. In brief, folic acid was added to the solution containing dendrimer, or ethylenediamine, and the mixture was incubated in a vibrating water bath at 25 °C for 24 h. The solution was then centrifuged and the supernatant was diluted to the proper concentration for UV characterization. The absorbance of the solution at the characteristic wavelength of 280 nm was measured using a UV-visible spectrophotometer (TU-1900) and 10 mm path-length quartz cells. For the calculation of solubility, 0.05 mg/mL folic acid in 0.1 mol/L acetate buffer (pH 5.8) was prepared and diluted to several sample solutions of certain concentration (0.001–0.03 mg/mL). The absorbance of the sample solutions at 280 nm was measured and plotted to make the calibration curve of folic acid.

3. Results and discussion

3.1. PAMAM dendrimers enhance the solubility of folic acid at pH 11

The solubility of folic acid under the basic condition was first investigated in the presence of G0–G2 PAMAM dendrimers. Because of the low pKa values (4.65 and 6.75) [17], the two carboxylic acid groups in folic acid are entirely deprotonated and negatively charged at pH 11. The solubility of folic acid without any enhancer compound was determined to be ~2 mg/mL, indicating that the ionization of folic acid plays an important role in its solubilization process. When PAMAM dendrimers are introduced, the dissolution of folic acid in water is significantly enhanced (Fig. 2a). For example, the solubility increases to ~20 mg/mL in the presence of 1 mmol/L G0 PAMAM dendrimer and to ~135 mg/mL with 10 mmol/L G2 dendrimer. The solubility of folic acid increases with the concentration of dendrimer in all three generations, which is consistent with the literature [6–9]. At the higher concentration, there are more dendrimer molecules in the solution that can interact with folic acid, help the solvation of folic acid and in turn enhance its solubility. Meanwhile, the solubility of folic acid