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Developing novel strategies for the functionalization of core–shell magnetic nanoparticles with folic acid derivatives

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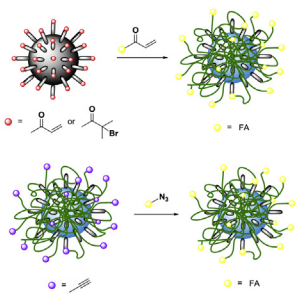
HIGHLIGHTS

- Synthesis of folic ester functionalized magnetite nanoparticles.
- The amine site of the folic acid was employed for functionalization.
- ATRP polymerization of the FA monomer was carried out at the nanoparticle surface.
- Characterization of the magnetic nanoparticles by FT-IR, TEM, XPS.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we explore the development of new synthetic strategies leading to the formation of folic acid di-ester functionalized iron oxide-based magnetic nanoparticles. For this, initially, folic acid di-ester was converted into an acrylamide monomer through amidation of the primary amine site. Interestingly, esterification with a different acid chloride molecule carrying a long alkyl chain terminated with an azide group resulted into amidation of the secondary amine site. In a different set of experiments, magnetite nanoparticles decorated with a polymerization initiator, an acrylamide monomer, or a terminal alkyne group, were prepared. These folic acid and magnetic nanoparticles building blocks could then be combined in different ways to give rise to the targeted materials. In the first scheme, folic acid-based monomer could be polymerized through the initiating sites located at the surface of the magnetic nanoparticles. Alternatively, a random copolymerization between the folic acid-based monomer was carried out with the monomeric sites stabilizing the nanoparticles. Finally, the alkyne functionalities of the nanoparticles were coupled with the azide moieties of the folic acid derivatives. Thus, three different synthetic strategies were established for the formation of folic acid di-ester functionalized core–shell magnetic nanoparticles.

1. Introduction

Folic acid (FA) is well known for its biological recognition capabilities [1]. It is for this reason that magnetic nanoparticles have often been functionalized with this biologically active motif [2–11]. Such functionalized nanoparticles can bind to certain cancer cells

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over expressing the folate receptor [12]. It is not surprising, therefore, that a number of magnetic nanoparticle modification schemes with FA have been established. For example, magnetic nanoparticles have been modified with amino, carboxy, or azido groups and FA functionalization is achieved through amidation [2–6] esterification [7] or click chemistry reactions [8–11]. Functionalization with FA as a carboxylic component, however, faces the problem that either or both of the two existing carboxylic acid moieties, located at the glutamate site (α- and γ-isomers), may react while only one of them is involved in the biorecognition mechanism [13]. Therefore, the resulting materials may contain a mixture of active and inactive folate sites [14–17]. From a different perspective, low solubility of folic acid in all common solvents hampers its applications in the nanoparticle modification protocols. On the other hand, di-alkyl esters of folate exhibit high solubility and gained pharmaceutical importance [18]. It is surprising to note, therefore, that such dialkyl ester derivatives of folic acid have not been examined for the functionalization purposes of magnetic nanoparticles.

In this work, we investigate the potential of such highly soluble derivatives of folic acid for the functionalization of magnetic nanoparticles. To achieve this goal, a new approach is established, using the aromatic primary or secondary amine instead of the acid moieties of the folic acid for anchoring the reactive site. The reactive sites included either a polymerizable acrylate or an azide functionality. On the magnetic nanoparticle side, polymerization initiators or an alkyne functionality were chosen as reactive sites. Therefore, nanoparticle functionalization could be achieved either by polymerizing the folate monomer or by carrying out a Cu-catalyzed azide-alkyne cycloaddition reaction (CuAAC) at the particle shell. In this way, folate functionalized core–shell magnetic nanoparticles could be prepared with synthetic ease. To the best of our knowledge, this is the first report in which the secondary amine of the FA is shown to be a suitable anchoring site for further functionalization processes.

2. Experimental

Chemicals were purchased from Aldrich and Acros unless noted otherwise. The starting materials **1a** [19], **4** [20], **7** [21], **12** [21] were synthesized using the literature procedures. Silica gel 60 (0.04–0.063 mm, Acros) was used for preparative column chromatography. High-resolution mass spectra (ESI) were obtained with a Thermo Finnigan LTQ-FT-ICR-MS using MeOH as a solvent. NMR spectra were recorded on a 500-MHz spectrometer (Bruker Avance III spectrometer), chemical shifts (δ) are reported in ppm relative to TMS.

The morphology of functionalized magnetic nanoparticles was determined by 1010 JEOL transmission electron microscope. FT-IR spectra were obtained on a JASCO FTIR 610 spectrophotometer. The magnetic measurements were performed at room temperature using a Vibrating Sample Magnetometer Cryogenics. Dynamic light scattering (DLS) measurements were carried out with a Brookhaven Instruments Corp. goniometer and laser light scattering system. Acquisition time was set at 90 s, a laser radiation wavelength of 632.8 nm was used and the angle at which data acquisition was performed is 90°. The chemical surface analysis for the functionalized magnetic nanoparticles was obtained using X-ray Photoelectron Spectroscopy (XPS) technique. XPS spectra were recorded using a SPECS spectrometer, equipped with a dual-anode X-ray source Al/Mg, a PHOIBOS 150 2DCCD hemispherical energy analyzer and a multi-channeltron detector. The pressure inside the measurement chamber was maintained constant at about 1×10^{-9} torr. The sample, as a colloidal suspension in methanol, was dried in successive layers on indium foil stacked on a wolfram sample holder. Irradiation was made with an $Al_{K\alpha}$ X-ray source (1486.6 eV) operated at 200 W. The XPS survey spectra were recorded at 30 eV pass energy, 0.5 eV/step. The high resolution spectra for individual elements were recorded by accumulating 10–15 scans at 30 eV pass energy and 0.1 eV/step. Data analysis and experimental curve fitting was performed using Casa XPS software with a Gaussian–Lorentzian product function and a non-linear Shirley background subtraction.

2.1. Synthesis of folic acid dimethyl ester acrylamide (*2a*)

To a stirred solution of folic acid dimethyl ester **1a** (938 mg, 2 mmol) in DMF (20 ml) was added Et_3N (276.6 μl, 202 mg, 2 mmol). The solution was cooled at 0 °C and acryloyl chloride (148 μl, 163 mg, 1.8 mM) was slowly added. The mixture was stirred at room temperature overnight and evaporated. Further purification attempts led to polymerization. Yield 64%.^{1}H NMR (DMSO-d_6): δ = 1.96 (m, 1H), 2.05 (m, 1H), 2.42 (m, 2H), 3.56 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 4.37 (m, 1H), 4.49 (s, 2H), 5.87 (dd, 1H, J = 1.8 Hz, J = 10.3 Hz, vinyl), 6.08 (dd, 1H, J = 10.3 Hz, J = 17.3 Hz, vinyl), 6.25 (dd, 1H, J = 1.8 Hz, J = 17.3, vinyl), 6.64 (d, 2H, J = 8.8 Hz, CH-Ar), 7.64 (d, 2H, J = 8.9 Hz, CH-Ar), 8.65 (s, 1H) ^{13}C NMR (DMSO-d_6): δ = 25.7, 29.9, 35.8 (−CH_2−CH_2−), 51.3 (CH), 51.7, 51.8 (CH_3), 120.8 (Cq Ar), 127.9, 129.0, 129.4, 130.7, 148.4, 150.8, 153.7 (C vinyl and Ar), 162.3 (C=O), 166.4, 166.9 (NH−C=O), 172.6, 172.7 (O−C=O). HRMS (APCI) calculated for $C_{24}H_{26}N_7O_7$ $[M+H]^+$: 524.1888, found: 524.1886.

2.2. Synthesis of folic acid dibenzyl ester (*1b*)

Folic acid (3.0 g, 6.8 mmol) was added to a stirred solution of methanesulfonic acid (3.0 g, 2 ml, 31.25 mmol) in a mixture of benzyl alcohol (50 ml) and DMF (50 ml). The mixture was stirred at room temperature for 2 days and then DMF was removed. The remaining solution was treated with chloroform (50 ml) and methanol was slowly added under very slow stirring. The formed precipitate was filtered at normal pressure and recrystallized from methanol. Yield 67%.^{1}H NMR (DMSO-d_6): δ = 2.02 (m, 1H), 2.12 (m, 1H), 2.48 (m, 2H), 4.46 (m, 1H, overlapped), 4.49 (s, 2H, overlapped), 5.06 (s, 2H, benzylic), 512 (br s, 2H, benzylic), 6.64 (d, 2H, J = 8.7 Hz, CH-Ar), 7.32 (m, 10H, CH-Ar), 7.65 (d, 2H, J = 8.7 Hz, CH-Ar), 8.32 (d, 1H, NH), 8.65 (s, 1H). ^{13}C NMR (DMSO-d_6): δ = 25.7, 30.1, 45.8 (−CH_2−), 51.3 (CH), 65.5, 65.8 (CH_3), 111.2 (CH_2), 120.9 (Cq Ar), 127.7–129.1 (C Ar), 136.0, 136.1, 148.5 (C Ar), 150.9 (C=O), 153.7, 166.6 (NH−C=O), 172.0, 172.1 (O−C=O). HRMS (APCI) calculated for $C_{33}H_{33}N_7O_6$ $[M+H]^+$: 622.2409, found: 622.2410.

2.3. Synthesis of folic acid dibenzyl ester acrylamide (*2b*)

The product was obtained in the same way as **2a** using folic acid dibenzyl ester **1b**. Yield − crude product 95%.^{1}H NMR (DMSO-d_6): δ = 1.97 (m, 1H), 2.05 (m, 1H), 2.41 (m, 2H), 4.37 (m, 1H), 4.48 (s, 4H), 4.53 (s, 2H), 5.87 (dd, 1H, J = 1.7 Hz, J = 10.3 Hz, vinyl), 6.08 (dd, 1H, J = 10.3 Hz, J = 17.3 Hz, vinyl), 6.25 (dd, 1H, J = 1.7 Hz, J = 17.3, vinyl), 6.65 (d, 2H, J = 8.6 Hz, CH-Ar), 7.30 (m, 10H), 7.66 (d, 2H, J = 8.7 Hz, CH-Ar), 8.31 (d, 1H, 7.3 Hz, NH), 8.70 (s, 1H).

2.4. Synthesis of azide functionalized folic acid esters (*3a*, *3b*)

To a stirred solution of folic acid dimethyl ester **1a** or **1b** (938 mg, 2 mmol) in DMF (20 ml) was added Et_3N (276.6 μl, 202 mg, 2 mmol). The solution was cooled at 0 °C and an excess of crude 6-azidohexanoic acid chloride (702 mg, 4 mM) was slowly added. The mixture was stirred at room temperature overnight and evaporated. The crude mixture was separated by column chromatography with 3–7% ethanol in dichloromethane.

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