Communication

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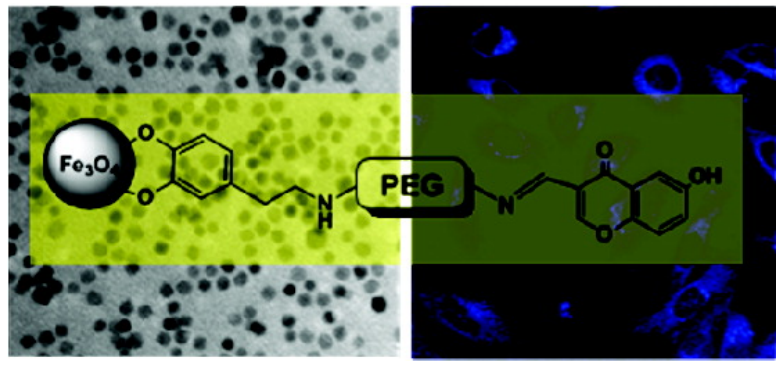
J. Am. Chem. Soc., **2008**, 130 (44), 14436-14437 • DOI: 10.1021/ja806519m • Publication Date (Web): 08 October 2008



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Published on Web 10/08/2008

pH Controlled Release of Chromone from Chromone-Fe_3O_4 Nanoparticles

Baodui Wang,[†,‡] Chenjie Xu,[†] Jin Xie,[†] Zhengyin Yang,[*,‡] and Shouheng Sun[*,†]

Department of Chemistry, Brown University, Providence, Rhode Island 02912, and College of Chemistry and Chemical Engineering and State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, P.R. China

Received August 21, 2008; E-mail: yangzy@lzu.edu.cn; ssun@brown.edu

Magnetic Fe_3O_4 nanoparticles (NPs) are promising as drug delivery vehicles for both diagnostic and therapeutic applications.[1] The key to achieving these dual applications is that the drug-Fe_3O_4 NPs are stable in a biological circulation system, readily interact with cells or other biological units of interest, and are capable of releasing the drug once the selected targeting is realized.[2] Currently, drug-Fe_3O_4 NP conjugates are made either by embedding the drug in the hydrophobic media in the double-layer coating of Fe_3O_4 NPs[3] or by incorporating both drug and Fe_3O_4 NPs in the SiO_2 matrix.[4] Although the conjugates prepared from these methods show enhanced dispersion stability, they have a hydrodynamic diameter of 150 nm or larger. Such large NP delivery systems may have very limited extravasation ability and may be subject to easy uptake by the reticuloendothelial system (RES),[5] unsuitable for target-specific delivery applications. Recently we reported that Fe_3O_4 NPs coated with dopamine (DPA) and COOH-terminated polyethylene glycol (PEG) are stable in cell culture media against macrophage cell uptake.[6] The hydrodynamic sizes of the NPs are tuned by the length of the PEG molecules. These PEG-DPA-Fe_3O_4 NPs offer an ideal platform for drug coupling and delivery.

Here we report that 6-hydroxy-chromone-3-carbaldehyde (**1a**) can be readily coupled to these PEG-DPA-Fe_3O_4 NPs via a Schiff-base bond, as shown in Figure 1A, and released via a pH controlled manner. Chromones are a group of naturally occurring compounds containing core structure of benzopyranone and have been shown to be antifungal, antiviral, antihypertensive, and anticancer agents.[7] However, their low solubility and short blood circulation time limit their usage for efficient therapeutic applications. We demonstrate that **1a** coupled to **1c** shows a dramatic increase in solubility from less than 2.5 for free chromone to 633 μg/mL for **1d**. **1d** is stable in neutral pH condition but releases free chromone very quickly in pH lower than 6, and inhibits HeLa cell proliferation more efficiently than the free chromone. This, plus the fluorescent chromone and superparamagnetic Fe_3O_4 NPs, renders **1d** a powerful multifunctional delivery system for diagnostics and therapeutic applications.

Fe_3O_4 NPs coated with a layer of oleate/oleylamine were synthesized through the decomposition of $Fe(acac)_3$ with the core size around 12 nm (Figure 1B).[8] The as-synthesized NPs were made biocompatible by replacing oleate/oleylamine with DPA-PEG (**1b**), giving **1c**. **1a** was loaded onto **1c** via the formation of a Schiff-base bond.[8] **1d** was readily dispersed in water with chromone solubility reaching 633 μg/ml,[8] equal to ~140 chromone molecules per Fe_3O_4 NP. The Fe_3O_4 core in **1d** (Figure 1C) is similar to what is seen in Figure 1B, indicating no NP morphology change in the surfactant exchange process.

The Schiff-base bond is biodegradable via hydrolysis, and the process can be accelerated at low pH conditions.[9] To examine the

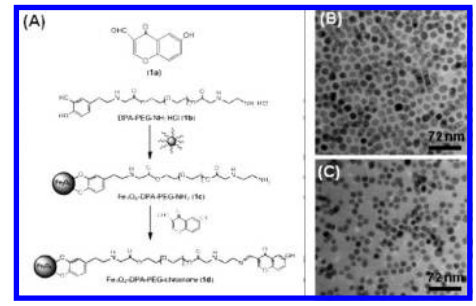

Figure 1. (a) Structure of chromone (**1a**) and the schematic illustration of the coupling between chromone and a Fe_3O_4 NP; TEM images of (b) the as-synthesized 12 nm Fe_3O_4 NPs from the hexane dispersion and (c) the chromone modified Fe_3O_4 NPs (**1d**) from water.

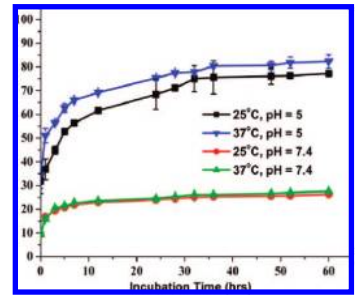

Figure 2. The percentage of the chromone released in PBS + 10% FBS from **1d** with the incubation time under different pH values and temperatures in PBS.

pH controlled release of chromone in **1d**, we put the conjugate in the dialysis bag and incubated at different temperatures in the phosphate-buffered saline (PBS) plus 10% fetal bovine serum (FBS) with pH at 5 and 7.4. The detailed pH-dependent release in different buffers with pH ranging from 3 to 9 is given in the Supporting Information, Figure S4. The released chromone was quantified through its fluorescent signal.[8] Figure 2 shows the percentage of chromone released from **1d** at different pH values and temperatures. It can be seen that a few chromone are detached from **1d** in the PBS of pH = 7.4 at both 25 and 37 °C culture conditions. However, in the PBS of pH = 5, the conjugate exhibits a drastic increase in free chromone and the higher incubation temperature (37 °C) results in higher chromone concentration in the solution. The slight increase in chromone release at 37 °C in pH = 5 indicates that the Schiff-base bond is subject to faster hydrolyzation at higher temperature. The hydrodynamic size of **1d** is decreased in pH = 5, but those in pH = 7.4 are stable, as shown in Figure S5. This proves that chromone is released from **1d** at low pH but is stable in the conjugate at pH = 7. From Figure S5, one can also see that **1c** are stable in the incubation conditions and show no statistical hydrodynamic size change over the incubation time. The measured size

[†] Brown University.
[‡] Lanzhou University.

10.1021/ja806519m CCC: $40.75

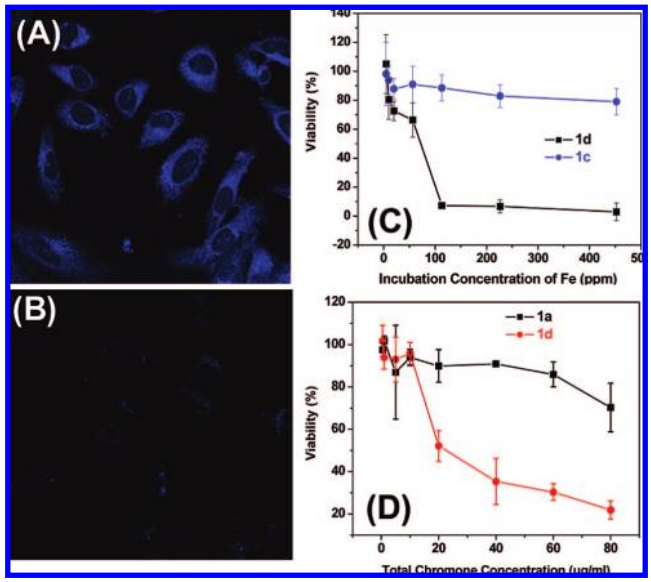

Figure 3. Fluorescent images of HeLa cells after incubated with (A) **1d** and (B) **1a** for 1 h; and viability of HeLa cells in the presence of (C) total iron concentration and (D) total chromone concentration.

increase from ~60 to ~110 nm in the presence of FBS is attributed to the adsorption of FBS onto the NP surface as reported previously.[10]

The increased solubility of chromone present in **1d** led to the enhanced uptake of **1d** by HeLa cells. Figure S5 shows that at the same iron concentrations (7 μg/ml), more **1d** than **1c** are taken up by HeLa cells. Similar uptake enhancement is also observed for **1d** over **1a**. Figure 3 panels A and B are fluorescent images of the HeLa cells after their incubation with **1d** and **1a** in the same chromone concentration at 15 μg/ml. Because of the high chromone solubility in **1d**, there exist more chromone molecules in solution interacting with HeLa cells, leading to the enhanced uptake and brighter image of the cells in Figure 3A. In contrast, the free chromone has very low solubility and with the same total amount of chromone added, the majority of the free chromone stays in the solid form and can be separated by centrifugation (8000 rpm). As a result, there is only small amount of free chromone in solution interacting with the cells, resulting in fewer uptakes and much darker fluorescent image (Figure 3B).

The enhanced uptake of **1d** leads to high toxicity to the HeLa cells. Figure 3 panels C and D are the HeLa cell viability data under the same iron (Figure 3C) concentration or the same amount of chromone added to the cell culture medium (Figure 3D). It can be seen that both **1c** and **1a** have very limited toxicity to HeLa cells as **1c** has no chromone and **1a** has the very low solubility in the buffer, preventing its uptake by the cells. In contrast, **1d** are highly toxic with majority of the cells destroyed at ~100 ppm iron concentration or at ~40 μg chromone/ml. This high toxicity of **1d** to the HeLa cells comes likely from their enhanced uptake by the HeLa cells and the controlled release of free chromone from **1d** in the low pH cellular environment. The IC50, the half-maximal (50%) inhibitory concentration (IC), of **1d** can be extracted from Figure 3D to be ~21 μg/ml. Note that in the current experimental conditions, we could not get IC50 for **1a**, **1b**, and **1c** because of their low solubility/low toxicity to HeLa cells. It should also be mentioned that the detailed toxicity mechanism for the chromone−Fe_3O_4 NPs is still unclear. However, from the fact that the free chromone can be released in a low pH environment and that the endosome/lysosome within a cell have the low pH's (<6), we can reasonably assume that the toxicity arises from the release of the free chromone from the chrome−Fe_3O_4 conjugate in the endosome/lysosome of the HeLa cells.

The current work demonstrates that chromone coupled to PEG-DPA-Fe_3O_4 NPs shows a dramatic increase in chromone solubility in cell culture medium from less than 2.5 to 633 μg/mL, and the free chromone can be released in a controlled manner at low pH conditions. The high chromone solubility in the chromone-Fe_3O_4 conjugate leads to the enhanced chromone uptake by HeLa cells, and as a result, the chromone shows a much more efficient inhibition to the HeLa cell proliferation. Although it is unclear how powerful/useful of the chromone molecule is as an anticancer agent, its high solubility achieved in the chromone-Fe_3O_4 NP conjugate should allow for more tests on this front. Furthermore, the reported concept should be readily extended to the synthesis of other anticancer drug−Fe_3O_4 NP conjugates. With controlled drug release only in the intercellular low pH conditions, such drug−Fe_3O_4 conjugates may offer much needed efficacy in successful cancer therapy.

Acknowledgment. The work was supported by Grant NIH/NCI 1R21CA1285-1. B.W. thanks the support from National Natural Science Foundation of China (20475023).

Supporting Information Available: Nanoparticle/surfactant synthesis and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA806519M