Tetrahedron 70 (2014) 991–995

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of a self organizable curcumin derivative and investigation of its interaction with metals in 100% aqueous media

Joshna Chittigori [a,b], Abhishek Kumar [a,c], Lian Li [d], Sammaiah Thota [a], Akshay Kokil [a], Lynne A. Samuelson [d], Daniel J. Sandman [a,b,*], Jayant Kumar [a,c,*]

[a] Center for Advanced Materials, University of Massachusetts Lowell, Lowell, MA 01854, USA
[b] Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854, USA
[c] Department of Physics, University of Massachusetts Lowell, Lowell, MA 01854, USA
[d] US Army Natick Soldier Research, Development and Engineering Center, Natick, MA 01760, USA

ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 18 November 2013
Accepted 20 November 2013
Available online 27 November 2013

Keywords:
Synthesis
Curcumin
Bis PEGylated curcumin
Self organization
Nanoparticles
Absorption and fluorescence
Metal interactions

ABSTRACT

We report the synthesis of a self organizable and water dispersible bis PEGylated (bis polyethyleneglycolated) curcumin (**1**) using an efficient synthetic method. Compound **1** forms nanoparticles on the order of 90 nm in water. Even though it forms nanoparticles, compound **1** interacts strongly with metal ions, Al^{+3}, Cu^{+2}, and Hg^{+2}. Fluorescence and absorption spectroscopic techniques were employed to investigate the metal interactions of these nanoparticles. Al^{+3} and Cu^{+2} ions interact with compound **1** leading to enhancement and dramatic quenching in the fluorescence of the latter in 100% water, respectively. Addition of Hg^{+2} ions to compound **1** in water produces a ratiometric change in the absorption spectra of the nanoparticles. Although PEGylated curcumin derivatives were reported in the past, there have been no reports on their self organization into nanoparticles and extensive studies on their metal interaction properties in 100% water using its fluorescence and absorption properties.

1. Introduction

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-dione) is a phytochemical, extracted from *curcuma longa*. It has been used as a dietary spice and traditional medicine for centuries in Asia. Recent research has shown that this compound exhibits promising biological and pharmacological properties.[1] It has been used to treat symptoms of Alzheimer's disease and diabetes, and as a cancer chemo preventive, anti-inflammatory, and antioxidant. In addition, due to the presence of the β-diketone at the center of the molecule, it can chelate to many metal ions.[2] It has been suggested that accumulation of toxic metals, copper, mercury, and aluminum in a human body promotes disorders, such as Alzheimer's disease, and oxidative stress.[3] It is believed that the metal chelating property of curcumin is one of the reasons for its ability to reduce the symptoms of these diseases.[4] In addition curcumin also has interesting optical properties as it absorbs and emits strongly in the visible spectral region. As demonstrated in our previous work, it is two-photon active and can be employed for one and two-photon imaging applications.[5] However, curcumin is a hydrophobic molecule and not soluble in water. Due to its incompatibility with water, bioavailability and absorption are poor hindering its success in therapeutic applications.[6] The most widely employed methods to improve curcumin's water solubility are encapsulation of curcumin in polymer nanoparticles, usage of curcumin–PEG polymers or employment of mixtures of organic solvents, such as dimethylsulfoxide and water to dissolve curcumin.[7] These methods offer significant improvement in the efficacy of curcumin compared to curcumin in water by itself. However, the disadvantages with these methods include lack of consistency in loading of the nanoparticles, concentration of curcumin in the polymers and toxicity of organic solvents.

To circumvent these problems, we report here the design and synthesis of a curcumin derivative that self organizes into nanoparticles in water and forms stable dispersions. The synthesized curcumin derivative is not a polymer, but a small molecule. This method not only improves curcumin's water compatibility, but also has the potential to enhance its retention time, bioavailability, and absorption avoiding loading problems and use of toxic solvents. In order to achieve this goal, water soluble, non-toxic, and biocompatible polymer, PEG, has been chosen to covalently functionalize curcumin.

* Corresponding authors. Tel.: +1 (978) 934 3788; e-mail address: Jayant_Kumar@uml.edu (J. Kumar).

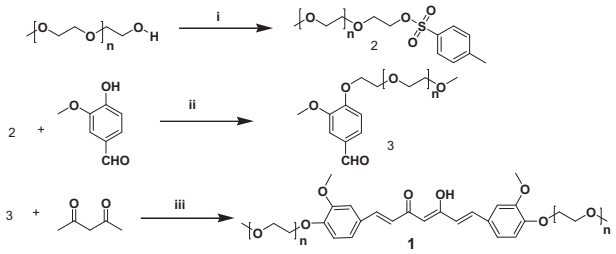
0040-4020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2013.11.071

Although there have been reports on chemically PEGylated curcumin derivatives in the past, they have been reported as water soluble.[7c,8] However, in our work, we observed self organization of compound **1** to form nanoparticles at concentrations as low as 40 μg of it in 1 mL of water. Titration experiments were carried out at the same concentration. In addition, even though compound **1** forms nanoparticles in water, it efficiently interacts with metals and undergoes significant changes in its absorption and emission characteristics. To the best of our knowledge, this observation has not been reported so far.

In contrast to most of the high molecular weight PEGylated curcumin derivatives reported in the past,[7c,9] we have functionalized curcumin with low molecular weight PEG. This results in better control of the curcumin to PEG ratio in the final product and also simplifies its characterization. We have chosen PEG with average molecular weight of 350 to make curcumin sufficiently amphiphilic to form nanoparticles in water. PEG was attached at both phenolic positions of curcumin using easy, straightforward, and efficient synthetic method. The synthesized two new compounds, intermediate PEGylated vanillin (3) and final product compound **1**, were purified just by solvent extraction method.

2. Results and discussion

Synthesis of compound **1** was achieved in three steps as shown in Scheme 1. PEGylation of curcumin can be achieved directly by reacting curcumin with tosylated PEG. However, this method yields both bis and mono PEGylated curcumin, which are difficult to separate. Therefore, PEGylated vanillin (3) was used as a starting material to synthesize bis PEGylated curcumin. This method produces compound **1** in high yield with high purity employing simple purification techniques. Chemical characterization of intermediates and compound **1** was performed using proton nuclear magnetic resonance (^{1}H NMR), carbon-13 NMR (^{13}C NMR), electrospray ionization mass (ESI-MS), and Fourier transform infrared (FTIR) spectroscopic techniques (Fig. S1–S8).



Scheme 1. Synthesis of compound **1** (i) TsCl, NaOH, THF/H_2O, 0 °C, 4 h, (ii) K_2CO_3, CH_3CN, reflux, 12 h, (iii) B_2O_3, $B(OCH_2CH_2CH_2CH_3)_3$, butyl amine, DMF, 75 °C, 10 h.

^{1}H NMR (Fig. S2) and ^{13}C NMR (Fig. S3) spectra of intermediate 3 showed characteristic peaks of aldehyde at 9.82 ppm and 190.8 ppm with peaks for PEG from 3.00 to 4.5 ppm and from 50 to 75 ppm, respectively. The ^{1}H NMR (Fig. S4) of the compound **1** showed 3 olefinic peaks that are characteristic of curcumin at 7.60–7.64 ppm, 6.50–6.54 ppm, and 5.84 ppm. ^{13}C NMR (Fig. S5) further confirmed the final product with the carbonyl peak at 183.2 ppm and no aldehyde peak at 190.8 ppm. Electrospray ionization mass spectra of intermediate 3 (Fig. S6) and compound **1** (Fig S7) showed molecular ions signals, $[M+Na]^+$, with 44 mass units difference. This difference is attributed to OCH_2CH_2, a signature unit of PEG chains. In FTIR spectra (Fig. S8), the carbonyl peaks of intermediate 3 and compound **1** appeared at 1680 cm^{-1} and 1623 cm^{-1}, respectively. The shift in stretching frequency of C=O from 1680 cm^{-1} (intermediate 3) to 1623 cm^{-1} (compound **1**) is because of the extension of conjugation in curcumin of compound **1**. C–O stretching frequencies appeared at 1098 cm^{-1} and 1132 cm^{-1} in intermediate (3) and compound **1**, respectively. It is evident that the intensity of C–O stretching frequency in compound **1** is much stronger than in intermediate 3.

Compound **1** is highly soluble in organic solvents, such as chloroform, methanol, and tetrahydrofuran. However, when 40 μg of compound **1** was mixed with 1 mL of water, although it appeared soluble to the naked eye, investigation by dynamic light scattering (DLS) revealed the formation of nanoparticles with an average size of 90 nm as shown in Fig. 1a. Cryo scanning electron microscopy (cryo SEM) was also performed to confirm the size of the nanoparticles (Fig. 1b). At concentrations significantly below 40 μg/mL, because of lower number of particles, the scattering intensity and signal to noise ratio are considerably reduced. Hence, a reliable estimation of particle formation is not possible.

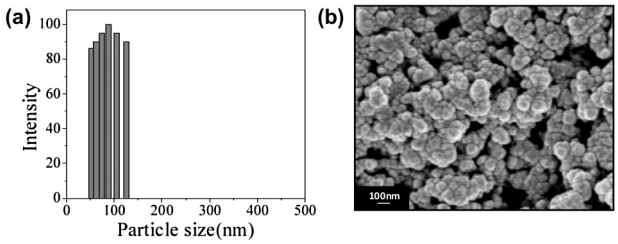


Fig. 1. a. Particle size distribution measured by DLS, and b. cryo SEM image of the nanoparticles of 40 μg of compound **1** in 1 mL of water.

The formation and stability of these nanoparticles can be explained based on Gibbs free energy. Compound **1** possesses both hydrophobic curcumin and hydrophilic PEG units. The hydrophobic curcumin units can stack together through π–π interactions in water, whereas PEG units can interact with water through hydrogen bonding. Both these interactions result in lowering of the Gibbs free energy. The π–π interactions between curcumin moieties lead to aggregation and formation of nanoparticles. However, formation of very large aggregates would result in a smaller entropic contribution to the decrease in Gibbs free energy. As regards with the structure of these nanoparticles, we speculate that PEG units on the corona (outer edges) of the nanoparticles hydrogen bond with water. Inside the core of the nanoparticles, the curcumin moieties tend to interact between themselves and the PEG units interact with themselves but are swollen by water molecules, which diffuse inside the nanoparticles. Such a structure would also explain the observation that metal ions can easily diffuse into the nanoparticles and interact with the curcumin moieties. Thus the interplay between the enthalpic and entropic contributions results in the formation of a relatively narrow distribution of colloidal nanoparticles with average size 90 nm under ambient conditions.[10] The stability of the nanoparticle dispersion is due to the fact that they have a surface charge. We have experimentally measured the zeta potential of these nanoparticle dispersion. The measured zeta potential is −45 mV, which is reasonably large and leads to stability of the nanoparticle dispersion.

Absorption and fluorescence spectra of compound **1** were recorded in chloroform, methanol, and water (Fig. 2a and b) and the results obtained are similar to the spectra of previously reported non-functionalized curcumin.[5,11] This indicates, functionalization of curcumin with PEG at phenolic positions does not affect its optical properties. In organic solvents, it exhibits only one absorption maximum around 420 nm (Fig. 2a), whereas in water, an additional peak around 360 nm appears. Because of the presence of the β-diketone group, curcumin can undergo keto–enol tautomerism. However, it exists preferentially in the keto–enol form