Food Research International 69 (2015) 202–208

Contents lists available at ScienceDirect

Food Research International

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

Modified curcumin with hyaluronic acid: Combination of pro-drug and nano-micelle strategy to address the curcumin challenge

Jinglei Li [a], Gye Hwa Shin [a], Xiguang Chen [b], Hyun Jin Park [a,*]

[a] School of Life Sciences and Biotechnology, Korea University, 5-Ka, Anam-Dong, Sungbuk-Ku, Seoul 136-701, Republic of Korea
[b] College of Marine Life Science, Ocean University of China, Qingdao, 266003, Shandong, PR China

ARTICLE INFO

Article history:
Received 12 November 2014
Accepted 23 December 2014
Available online 2 January 2015

Chemical compounds studied in this article:
Curcumin (PubChem CID: 969516)
Hyaluronic acid (PubChem CID: 24728612)
Gallic acid (PubChem CID: 370)
Dimethyl sulfoxide (PubChem CID: 679)

Keywords:
Curcumin
Hyaluronic acid
Nano-micelle
Antioxidant
Water solubility

ABSTRACT

Curcumin is well known for its pleiotropic activities such as anti-oxidant, anti-inflammatory and anti-tumor properties. But curcumin has extremely low solubility in aqueous medium which limits its potential applications in food and pharmaceutical industries. In this study, the combined pro-drug and nano-micelle strategy was attempted to address the limitation. We modified curcumin with hyaluronic acid (HA) based on the understanding that the hydrophilic polymer facilitates dissolution of curcumin in water. The amphiphilic curcumin derivative self-assembled into nano-micelle in water with the size around 100 nm and low PDI (<0.3) which indicated that it had narrow size distribution. FT-IR spectrum verified that curcumin was successfully conjugated onto the backbone of HA. On the other hand, curcumin was also loaded into the hydrophobic core of the nano-micelle which worked as carrier for it. Using this combined strategy, the water solubility of curcumin was increased more than 1100 times compared to the pure curcumin. The morphology characteristic of curcumin loaded nano-micelle was examined by TEM which confirmed that curcumin loaded nano-micelle has spherical shape and unified size distribution. Meanwhile, the antioxidant activities of the nano-micelle and the curcumin loaded nano-micelle were examined by DPPH radical scavenging assay and reducing power assay the results of which indicated that the antioxidant potentials were significantly enhanced. The pro-drug and nano-micelle combined method reported here may be promising to address the curcumin challenge such as low water solubility and low availability.

1. Introduction

Curcumin [(E,E)-1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-ione] is a natural phenolic compound isolated from the perennial herb *Curcuma longa* (turmeric) (Ramawat & Goyal, 2008). Turmeric has been consumed as food stuff and nutritional supplement in Indian and other oriental countries for thousands of years. Recently, numerous articles were released discussing the health beneficial effects of curcumin such as antioxidant, anti-inflammatory, anti-cancer and many others (Kuo, Huang & Lin, 1996; Gota et al., 2010; Menon & Sudheer, 2007; Surh & Chun, 2007). However, the pleiotropic activities of curcumin are not fully realized due to its low water solubility, low stability and low bioavailability when using conventional administrative methods (Onoue et al., 2010). In the past decades, researchers have explored several ways to solve these problems of curcumin. Among them, the nano-system and pro-drug strategies attracted much attention.

The nano-system, such as liposome, micelle, solid lipid particle and emulsion, has been developed to encapsulate curcumin and enhance its bioavailability (Yallapu, Jaggi & Chauhan, 2012). In our previous research, curcumin was loaded into chitosan-coated nano-liposome which showed higher mucin adsorption ability (Shin, Chung, Kim, Joung & Park, 2013). Lei Liu and co-workers studied the anti-tumor activity of a polymeric micelle loaded with curcumin (Liu et al., 2013). Many researchers also encapsulated curcumin into solid lipid particle formulation and reported the increased bioavailability and pharmacokinetics parameters (Kakkar, Singh, Singla & Kaur, 2011; Gota et al., 2010). Recently, silica nanoparticle was also studied as carrier to delivery curcumin (Tikekar, Pan & Nitin, 2013). Nevertheless, the need of novel nano-system which circumvents some of the inherent problems, such as complex preparation procedures, low stability, and low loading efficiency, are still there (Bansal, Goel, Aqil, Vadhanam & Gupta, 2011).

On the other hand, the pro-drug method also got great success to increase the water solubility of curcumin. Curcumin molecule has one hydroxyl group attached on each of the two benzene rings as depicted in Fig. 1. The hydroxyl group is highly reactive with carboxyl group as it tends to lose the proton under proper conditions. Hydrophilic polymers are usually used to conjugate with curcumin in the pro-drug approach. Synthetic polymers such as polyethylene glycol, poly (lactic acid) were reported as the hydrophilic moieties (Murphy, Tang, Van Kirk, Shen & Murdoch, 2012; Naksuriya, Okonogi, Schiffelers & Hennink, 2014). Even though the synthetic polymers achieved certain breakthrough, the safety concern about them always limits

* Corresponding author. Tel.: +82 2 3290 3450; fax: +82 2 953 5892.
E-mail address: hjpark@korea.ac.kr (H.J. Park).

http://dx.doi.org/10.1016/j.foodres.2014.12.045
0963-9969/

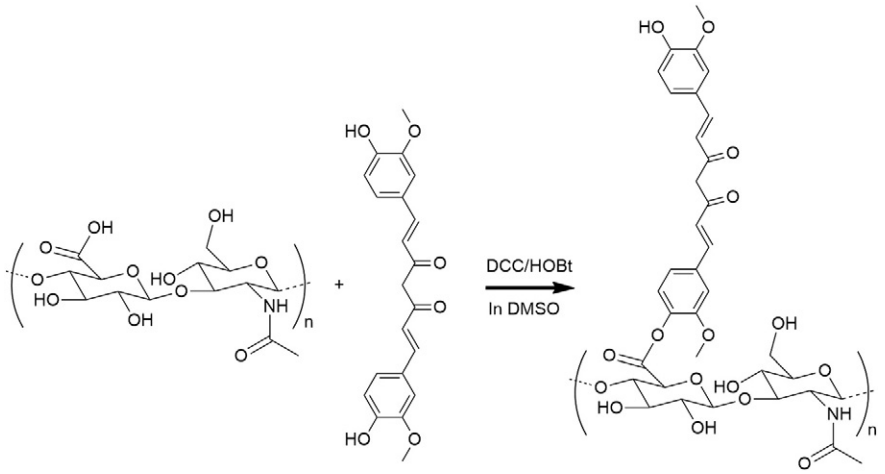

Fig. 1. Reaction between curcumin and HA in DMSO under the catalysis of DCC and HOBt. The reaction was carried out in room temperature for 24 h under gentle magnetic stirring.

their applications (Ishida, Wang, Shimizu, Nawata, & Kiwada, 2007). On the contrary, naturally occurred polymers show much better biodegradability and biocompatibility than their synthetic counterparts (Boztas et al., 2013). Among the naturally occurred polymers, hyaluronic acid (HA) owns particular features such as specific reaction with certain receptors (CD44 and RHAMM) which are over-expressed on the surface of many tumor cells (Misra et al., 2011; Eliaz, & Szoka, 2001). In fact, it has long been hypothesized that HA conjugated with anti-cancer drugs can work as the 'smart bullet' in the anti-cancer therapy (Misra et al., 2011). The carboxyl group on HA is usually the main target in the modification reaction because it can react with hydroxyl and amino groups of other compounds under the catalysis of carbodiimide reagents (Kong, Chen, & Park, 2011). In one recent publication, S. Manju and K. Sreenivasan reported the direct reaction between HA and curcumin in the DMSO/water (1:1) system (Manju & Sreenivasan, 2011). However, the reaction efficiency seems to be low as suggested by the results of their another recent released publication (Dey & Sreenivasan, 2014). So here, we conducted the similar reaction in pure DMSO which homogenously dissolved HA and curcumin. With the aim of combining the nano-micelle and pro-drug method, after the synthesis, we loaded curcumin again into the nano-micelle formed by the modified curcumin. The HA modified curcumin (HMC) dissolved in water with yellowish color and formed nano size micelle with low PDI. Curcumin was loaded into the core of the micelle using vortexing and sonication method and the loading capability was found to be high. After that, we also examined the antioxidant activity using DPPH radical scavenging and reducing power assay. The HMC not only increased the water solubility and antioxidant activity but also worked as carrier for curcumin which is promising to overcome its prime limitation.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt form with molecular weight of 10 kDa was provided by Kewpie Corporation (Tokyo, Japan). Curcumin with 98% purity was purchased from Acros Organics (New Jersey, USA). 1-Hydroxybenzotriazole hydrate (HOBt), 1,1-di-phenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu's reagent (FCR) and gallic acid were from Sigma-Aldrich (Missouri, USA). N,N′-Dicyclohexylcarbodiimide (DCC) was from TCI (Tokyo, Japan). Dimethyl sulfoxide (DMSO) was purchased from Duksan Pure Chemicals (Gyeonggi-do, Korea). Membrane tubing (MWCO: 2000) was obtained from Spectrum Laboratories (Rancho Dominquez, California, USA). All other reagents and solvents were of analytical grade and used as received.

2.2. Modification of curcumin with hyaluronic acid

The DMSO soluble HA was prepared according to our patented method (Kong, Chen & Cheng, 2013). After acidified by hydrochloric acid and washed with 75% ethanol, 95% ethanol and diethyl ether sequentially, HA sodium salt form was transformed to acid form. For the reaction, 40 mg (0.1 mM disaccharide repeat unit) HA was added to DMSO and dissolved under magnetic stirring for 1 h. DCC and HOBt dissolved in DMSO was then dripped into the HA solution. The molar ratio of DCC:HOBt:HA was kept at 1.5:1.5:1. The carboxyl group of HA was activated for 1 h before it was dripped into 0.2 mM curcumin MDSO solution. The reaction was carried out at room temperature for 24 h under gentle magnetic stirring and then the reaction solution was poured into 20 times volume of ethanol to precipitate the HA modified curcumin (HMC). Precipitated HMC was reclaimed by filtration and purified by dialysis against distilled water for 48 h. The yellow cotton-like HA modified curcumin was retrieved after freeze drying.

2.3. Characterization of HMC

2.3.1. FT-IR investigation

Varian 640-IR (California, USA) was employed to record the fourier-transform infrared (FT-IR) spectra of the HMC at the wavelength region between 400 and 4000 cm^{-1}. Samples were prepared using the KBr pallet method as reported by the previous publication (Kong, Chen & Park, 2011).

2.3.2. Measure the degree of substitution (DS)

Degree of substitution (DS) is an important parameter which reveals the reaction efficiency. In this study, DS was defined as the number of curcumin molecules per one hundred HA repeating disaccharide units and was calculated in accordance with the standard curve listed below. The standard curve was obtained by measuring the absorption of curcumin DMSO calibration solutions at 420 nm:

$$Y = 0.102 * X + 0.038,\ R^2 = 0.999$$

where Y is the absorption at 420 nm in DMSO, X is the concentration of curcumin in ppm.

2.3.3. Determination of the water solubility of HMC

To evaluate the water solubility of HMC, excess amount of sample was dissolved in water and stirred for 6 h in room temperature. The solution was centrifuged at 3000 rpm for 20 min at room temperature. Supernatant was filtrated through syringe filter unit (0.8 μm) to remove the undissolved part. Filtrate was lyophilized and the water solubility