Colloid and Interface Science Communications 25 (2018) 1–6

Contents lists available at ScienceDirect

Colloid and Interface Science Communications

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

Enhanced Antibacterial Activity of Curcumin by Combination With Metal Ions

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ARTICLE INFO

Keywords:
Polyphenol
Nanoparticles
Tannic acid
Metal coordination
Antibacterial

ABSTRACT

The rising bacterial infection severely threatens human health and has already become a worldwide issue. Safe, green and efficient antibiotics are urgently needed. Curcumin is well known as a natural and green food additive, and has effective biological and pharmaceutical activity. To overcome its hydrophobic disadvantage, here, tannic acid and metal coordination were used to coat curcumin nanoparticles (NPs) for the antibacterial application. On the one hand, the coating method produced a high drug loading content. On the other hand, the involvement of metal ions enhanced the bacterial inhibition efficiency compared to curcumin and metal ions alone. Especially for *S. aureus*, the minimum inhibitory concentration of copper containing complex NPs was 7.5 times lower than that curcumin alone. Thus, our research provides a natural and green antibiotics system to effectively prevent bacterial infections, and has potential to be widely produced in industry due to the naturally derived raw materials.

1. Introduction

Nowadays, with the abuse of antibiotics, drug-resistant bacteria are on the rise and can cause more and more severe bacterial infection, which seriously threatens human health [1–4]. As reported [5–9], the production of new kinds of antibiotics is slower than the occurrence of drug-resistant strains. Most of the antibiotics are deactivated towards bacteria with drug tolerance. Especially for the multidrug resistant bacteria [10], such as Methicillin-resistant *Staphylococcus aureus*, thousands of people are died with the infection. In order to reduce the occurrence of drug resistant bacteria, safe, green and efficient antibacterial agents are extremely urgent to be made. Especially the natural products have great potentials to be applied in the antibacterial fields.

Curcumin is a kind of polyphenol derivates, which can be widely abstracted from flavouring agent and plant *Curcuma longa* [11–15]. It is proved that curcumin has general biological and pharmaceutical activity, which arouses more researchers' interests. As a green and non-toxic nature medicine, it has promising application in oxidation resisting [15,16], anti-inflammatory [17], anti-cancerous [18,19] and anti-bacterial area [20–24]. Although the curcumin is found to own the good therapic efficiency and safety, it has not been approved to be a therapeutic drug in the market, which is due to the low bioavailability and poor solubility. Some pharmacokinetic studies found that low amount of curcumin could be detected in the blood plasma, and 75% of that would be excreted from body after oral administration [25]. Further investigation found that curcumin was not stable *in vitro* simulated physiological environment [26]. All the above mentioned disadvantages inhibited the extensive usage of curcumin. One effective way to solve the problem is to use carrier for the drug transfer. There are numerous methods to load hydrophobic drugs to improve the transition efficiency of curcumin to kill bacteria, such as using polymer micelles [24], liposome [16,18], mesoporous materials [27] and hydrogel [28]. For example, Liu et al. [24] fabricated a polymeric micelle to carry silver nanoparticles and hydrophobic curcumin for combinational antibacterial application. However, the above mentioned methods afforded the relatively low drug loading yield of about 5%, which greatly reduced the using efficiency of curcumin. Thus new fabrication method is needed to improve the curcumin loading content.

Tannic acid (TA) is also a natural polyphenol, which is more hydrophilic in contrast with curcumin [29–31]. Since Ejima et al. found that TA could form strong coordination films with metal ions on the surface of nanoparticles [32], the studies by using the complex on drug delivery system had been widely reported [33–36]. The coating method was extremely easy and rapid, and the resulting films were pH-dependent. For example, Shen et al. coated TA-Fe^{III} on the surface of hydrophobic anti-cancer drugs paclitaxel (PTX) by one step, which improved the hydrophilicity of PTX [37]. The formed NPs could easily accumulate in tumor cells and behave excellent antitumor activity.

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https://doi.org/10.1016/j.colcom.2018.04.009
Received 23 March 2018; Received in revised form 24 April 2018; Accepted 28 April 2018

However, the application in antibacterial field had not been studied by using this method. Here we used this one step strategy to fabricate a hydrophilic and stable NPs system for antibacterial application. Firstly, the curcumin nanoparticles were formed using a diluting method, and then TA and metal ions were added to cover on the NPs simultaneously. Silver ion was a powerful antibacterial agent compared to Fe^{III} and Cu^{II}. However, it was not chosen in our system to chelate with TA, which was due to not only the chemical and biochemical conversion, but also the reduction by TA to form silver nanoparticles [38–40]. On the one hand, the TA-metal coordination could improve the solubility and stability of curcumin NPs. On the other hands, the introduction of metal ions could enhance the anti-bacterial properties of curcumin. Especially for the system containing Cu^{II}, the anti *S. aureus* efficiency was 200 times higher than that of Cu^{II} only, and 7.5 times of curcumin only. The effective antibacterial properties afford the curcumin-TA-metal system a potential application in general and green anti-bacterial field.

2. Experimental Section

2.1. Materials

Curcumin and tannic acid were purchased from Adamas-beta Ltd., and used as received. $FeCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$ and Tween-80 were obtained from Chron Chemical Company (Chengdu, China). All aqueous solution were prepared with Milli-Q water (resistance > 18 MΩ/cm). Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification.

2.2. Characterization

2.2.1. Preparation of TA-Metal Coated Curcumin

In a typical preparation process [37], curcumin powder was dissolved in dimethyl sulfoxide (DMSO) at 40 mg/mL, and 5 μL of the concentrated solution was added to 975 μL water with slowly and under ultrasonication for 5 min. Following this 10 μL of TA solution (24 mM) and 10 μL of $FeCl_3$, $CuCl_2$ (40 mM) solution were added to the above dispersion of nanocores under constant ultrasonication, respectively. The solutions were then dialyzed (5 kD) against deionized water for 12 h to completely remove the DMSO, TA, metal ions and the NPs were thus obtained. The resultant nanoparticles were lyophilized for 24 h to obtain powders and then used for further test.

2.2.2. Particle Size and Morphology Analysis

The mean particle diameter of curcumin nanoparticles was measured by dynamic light scattering (DLS) (performed on Malvern Zetasizer ZS90), with the laser of 633 nm and 4 mW at 25 °C and the collection time set to be automatic. A scanning electron micrograph (SEM) of the aqueous dispersion was recorded on a Hitachi S4800 microscope by spreading the nanoparticle dispersion over a silicon chip and drying it under air.

2.2.3. Determination of Drug Loading Content

To determine the drug loading content (DLC) [41] of Curcumin-TA-metal complex NPs, the sample powders were first immersed into 5 mL ethyl acetate (EA) and a small number of HCl was added. After extraction, the absorbance of curcumin in the supernatant was measured by UV-2600 UV − visible spectrophotometer (Shimadzu, Japan) at 418 nm. While the amount of metal iron was determined by ICP-AES (Thermo Elemental, IRIS Advantage). The curcumin concentrations in the samples were obtained from the calibration curve, and the drug loading content was calculated according to the following formulas: DLC (%) = (the weight of curcumin in curcumin-TA-metal complex / the weight of curcumin-TA-metal complex NPs) × 100%.

2.2.4. Curcumin Release Study

The curcumin release of curcumin-TA-metal complexs were performed in phosphate buffer (10 mM, pH 7.4). Briefly, 2 mL of the curcumin-load NPs solution (at a final concentration of 100 μg/mL) was introduced into a dialysis bag (molecular weight cut off: 2 kDa) and then immersed in 20 mL of buffer solution containing Tween-80 (0.1% *w*/w) at 37 °C. At certain time intervals, 1 mL of the dialysis solution was taken out and extracted with EA and 1 mL fresh buffer solution was added. Then the curcumin concentration was determined from the absorbance at 418 nm using UV − visible spectrophotometer and the release experiments were conducted in triplicate.

2.2.5. Antibacterial Activity

E. coli (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) were used for antibacterial tests. First, a single colony of *E. coli* or *S. aureus* on the solid Luria − Bertani (LB) agar plate was transferred into 5 mL of liquid LB culture medium and grown 4–6 h at 37 °C. The bacterial suspension was then diluted with LB culture medium to 1×10^6 CFU/mL. The different kinds of NPs at predetermined concentration (0, 2.5, 5, 10, 20, 40, 80 μg/mL) were mixed with 75 μL of the diluted bacterial suspension at equal volume and incubated at 37 °C for 18–20 h. Finally, the OD_{600} value of the solution was recorded by a Varioskan Flash (ThermoFisher SCIENTIFIC). The bacterial viability was calculated using the following formula: Bacterial viability = T/C × 100%, where T is cfu/mL of the test sample and C is cfu/mL of the control. Samples treated with deionized water were used as controls and each assay was repeated three times.

2.2.6. Hemolysis Assay

Hemolysis assay was performed following the literature method with minor modification [42–45]. First, fresh rat blood cells were harvested by centrifuging (100 g for 5 min) and washed by 0.9% NaCl. Then the supernatant was discarded and the remaining red blood cells (RBCs) were resuspended in 0.9% NaCl to obtain an approximate 5% (by volume) suspension. After that, 0.5 mL of the RBCs suspension was mixed with an equal volume of different NPs at predetermined concentration and incubated at 37 °C for 1 h. The mixtures were then centrifuged for 5 min. RBCs suspensions incubated with 0.9% NaCl and DI water were used as negative and positive control, respectively.

3. Results and Discussion

The fabrication of TA-metal coated curcumin NPs was conducted in 2 steps, which was shown in Fig. 1. First, 5 μL of the curcumin parent solution in DMSO was slowly dropped to 975 μL MiliQ water to form NPs under sonication. Then TA and ions were added separately, and the sonication was kept for another 5 min. After dialysis for 12 h, the free TA and metal ions were removed, and curcumin-TA-metal complex were obtained, named as Cur@TA-Fe^{III} and Cur@TA-Cu^{II}. The formed NPs were hydrophilic and well-dispersed in water, with obvious Tydall effect. As shown in Fig. 2 (a), the solution of Cur@TA-Fe^{III} was brown, and the Cur@TA-Cu^{II} was with a yellow color. Both of them were stable in water, and no precipitation could be observed at least in a month. While, the curcumin NPs without any modification started to precipitate just after 15 min. The TA-metal coated curcumin could also be kept in water with relatively high stability even extracted by chloroform, a good solvent for curcumin. The zeta potential of curcumin during coating steps was characterized to evaluate the surface charge variation of the NPs, shown in Fig. 2b. Curcumin NPs had a zeta potential value of −13.63 ± 0.17 mV, which was due to the negative charged phenol groups. After coating with TA, the zeta potential decreased to −19.76 ± 0.70 mV due to the introduction of more phenol groups. As TA and metal ions were both added, the coordination film could be formed on the surface of curcumin NPs, and the zeta potential kept decreasing to −24.4 ± 0.08 mV and − 25.57 ± 0.52 mV for Cur@TA-Fe^{III} and Cur@TA-Cu^{II}, respectively. The low zeta potential might be attributed to the abundant phenol groups of TA on the outer layer of the complex. The size of Cur@TA-Fe^{III} and Cur@TA-Cu^{II} were