Original article

Peroxidase-like activity and antimicrobial properties of curcumin-inorganic hybrid nanostructure

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For the first time in this study, curcumin was utilized as an organic component reacting with Cu (II) ion (Cu²⁺) as an inorganic component for fabrication of curcumin based Cu hybrid nanostructure (Cu-hNs). We also systematically examined the catalytic effect towards guaiacol and antimicrobial activities of Cu-hNs towards fish pathogen bacteria. For the characterization of Cu-hNs, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDX) and Fourier transform infrared spectrometry (FT-IR) analysis were used. We claimed that hydroxyl group might react with Cu²⁺ in phosphate solution (PO₄³⁻) to form Cu-hNs. However, more uniform and spherical Cu-hNs were not seen owing to absence of more reactive functional groups like amine and carboxyl groups on structure of curcumin. In addition to our findings, synthesis of Cu-hNs were carried out in the various pH values to evaluate the effect of pHs on formation of Cu-hNs. The Cu-hNs exhibited remarkable catalytic activity through the Fenton reaction in the presence of hydrogen peroxide (H₂O₂) and effective antimicrobial activities against Gram-positive/negative fish pathogen bacteria. In this study, cheap and efficient synthesis of nanoflowers (NFs) using plant extracts is proposed for biomedical applications rather than expensive molecules such as amino acids and DNA.
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1. Introduction

Although various plant extracts have been received much more attention compared to DNA, protein and amino acids for synthesis of metallic nanoparticles (NPs) owing to their low price, easy availability and high stability in harsh conditions, determination of active compound acting as reducing and capping agent in extract and control on size and shape of NPs still remained as unsolved issues (Ocsoy et al., 2013, Akhtar et al., 2013, Mittal et al., 2013, Leng et al., 2016, Kizildag et al., 2019). Recently, plant extracts were utilized to form organic–inorganic hybrid nanostructures instead of synthesis of metallic NPs (İldız et al., 2017, Baldemir et al., 2017, Altinkaynak et al., 2019). In the formation of organic–inorganic hybrid nanostructures, the presence of some functional groups like hydroxyl, diol, amine and carboxyl groups may play a critical role by reacting corresponding metal ions.

Organic-inorganic nanostructures called “nanoflower” (NFs) was introduced for the first time by Zare and co-worker in 2012 (Ge et al., 2012, Zhu et al., 2013). In this an encouraging breakthrough, uniform nanoflowers using proteins/enzymes as organic parts and copper (II) ions (Cu²⁺) as inorganic parts were prepared and exhibited greatly enhanced catalytic activities and stabilities owing to the unique shape effect, synergistic effect between enzyme and metal ion and localized enzyme concentrations. Since its discovery to today, researchers used various commercial or isolated enzymes for synthesis of flower shaped nanostructures with different metal ions and investigated their enhanced catalytic activities and showed how they were used as promising biosensors. In the literature, there are studies synthesis of horseradish peroxidase-Cu NFs (Somturk et al., 2015), urease-Cu NFs (Somturk et al., 2016), bovine serum albumin-Cu NFs (Yılmaz et al., 2016), lactoperoxidase-Cu NFs (Altinkaynak et al., 2016), Turkish black radish peroxidase-Cu NFs (Altinkaynak et al., 2017).
and horseradish peroxidase-Fe NFs (Ocsoy et al., 2015). It is also reported that NFs can be used as sensors to determine the presence of hydrogen peroxide, phenol (Lin et al., 2014) bacteria (Ye et al., 2016) and glucose (Zhu et al., 2013).

Although a few non-protein incorporated hybrid nanostructures even NFs were reported and used as an alternative to enzymes (Ildiz et al., 2017, Baldemir et al., 2017, Altinkaynak et al., 2019). We introduced an elegant and systematic study for the first time by using curcumin as an organic component reacting with Cu (II) ion (Cu2+) as an inorganic component for the fabrication of curcumin-inorganic hybrid nanostructure (Cu-hNs). Curcumin (have polyphenol structure) is an active ingredient of Curcuma longa L. (turmeric) and has anti-cancer, anti-inflammatory, and antioxidant properties (Kocaadam and Sanlier, 2017). We also systematically examine the catalytic and antimicrobial activities of Cu-hNs towards guaiacol and fish pathogen bacteria, respectively. This study is thought to be a guide for cheap and effective hybrid nanostructure synthesis and biological applications.

2. Materials and methods

2.1. Preparation and characterization of Cu-hNs

For the synthesis of Cu-hNs, curcumin solution was used as an organic part for incorporating Cu2+. Briefly, 0.1 and 0.5 mg/mL curcumin were dissolved in distilled water in separate beakers. The curcumin and Cu2+ solutions (8x10^{-4} M copper sulfate pentahydrate) were mixed and vigorously vortexed in phosphate buffer (10 mM PBS, pH from 2 to 13) for 30 s and each mixture was incubated for 3 days at 4°C. After incubation, precipitates at the bottom of falcon tubes were collected and centrifuged with distilled water at 10,000 rpm (3 times). The precipitates were dried at 50°C and stored for characterization, antimicrobial, and peroxidase-like activity studies (Baldemir et al., 2017). Flower-like shaped, presence of Cu2+, and functional groups involved in synthesis were characterized by SEM images (ZEISS EVO LS10), EDX (ZEISS EVO LS10) and FT-IR analysis, respectively.

Fig. 1. SEM images of hybrid Cu-nanoflowers at pH 2 (a: Distribution of nanoflowers; b: Petals and diameter of nanoflowers).

Fig. 2. SEM images of hybrid Cu-nanoflowers at pH 3 (a: Distribution of nanoflowers; b: Petals and diameter of nanoflowers).
2.2. Catalytic activity of Cu-hNs

The catalytic activity of Cu-hNs was evaluated by using guaiacol as a model substrate. For the reaction, 1 ml of PBS (pH: 6.8), 22.5 mM H₂O₂ (1 ml), 45 mM guaiacol (1 ml) and 3 mg Cu-hNs were sequentially mixed. The absorbance of the product (oxidation of guaiacol) was also monitored at 470 nm using a UV–Vis spectrophotometer (Altinkaynak et al., 2019).

2.3. Antimicrobial activity of Cu-hNs

The antimicrobial activities of the synthesized Cu-hNs were performed against Gram-positive bacteria (Lactococcus garvieae) and Gram-negative bacteria (Aeromonas hydrophile, Vibrio para-haemolyticus). Firstly, the concentrations of microorganisms were standardized at 0.5 Mc Farland (approximately 1.5 × 10⁸ CFU/mL). The minimum inhibitory concentrations (MICs) of the Cu-hNs, against tested isolates, were measured in 96-well microtiter plates. Briefly, initial concentrations 100% then two-fold serial dilutions (10⁻⁴ to 10⁻⁸) of the Cu-hNs, each plate included a positive control (medium and tested bacteria) and negative control (only medium), afterward, 100 μL of tested bacterial suspension was inoculated to each well except negative control column and incubated at 37 °C for 24 h. After incubation, 10 μL of each test sample was spread on the surface of blood agar. Plates were incubated at 37 °C for 24 h and observed visually for microbial growth. All the microtiter assay were practiced in triplicate (Altinkaynak et al., 2019).

Fig. 3. Effect of pH on formation of hybrid nanostructures: a: Nanopetals synthesized at pH 5 medium; b: Nanostructures synthesized at pH 7 medium; c: Nanostructures synthesized at pH 9 medium; d: Nanostructures synthesized at pH 11 medium; e: Nanostructures synthesized at pH 13 medium.

Fig. 4. FT-IR analysis of hybrid Cu-nanoflowers.
3. Results and discussions

3.1. Characterization of Cu-hNs

In this study, flower-shaped organic–inorganic hybrid nanostructures were synthesized by cheap and effective method with interaction of curcumin and Cu++. The morphology of flower-shaped hybrid nanostructures synthesized in pH 2 and 3 of PBS solutions determined by SEM images shown in Fig. 1 and Fig. 2. The effect of different PBS pH values on flower-shaped Cu-hNs was recorded and shown Figs. 1-2a-e. It was observed that petals were clustered in the pH 2 and 3 medium and flower like shapes were formed (Figs. 1-2), whereas petal-like structures occur in the pH 5 medium and no flower shapes are formed (Fig. 3a). It was observed that no petal-like structures were formed under the different pH conditions and just nanocrystals were formed (Fig. 3b-e). The images obtained by SEM analysis clearly demonstrate that medium pH have a significant effect on the nanopetal formation, aggregation of nanopetals and the formation of flower-shaped hybrid nanostructures. Similar to our findings, Yan et al. (2005), reported that pH conditions had a significant effect on the control of morphology of magnesium hydroxide (Mg(OH)2) Cu-NFs. According to reports of Cui and Jia (2017), and Altinkaynak et al. (2016), kind of organic molecules and inorganic metals, medium pH and temperature are effect of morphology of Cu-hNs. This information confirms our study.

The structural properties of Cu-hNs were investigated by FT-IR and EDX analyzes. Functional groups that play an active role in Cu-hNs formation were identified by FT-IR analysis (400 cm⁻¹-4000 cm⁻¹). The diffraction peaks were detected at 556 cm⁻¹ and 811 cm⁻¹ were related to O = PO and C-H vibration, respectively (Fig. 4). The bands PO and P = O which indicated the presence of phosphate groups were detected between 964 and 1259 cm⁻¹. The stretching bands from at 1398–1623 cm⁻¹ correspond to NH2 groups. The peaks observed at 2993 cm⁻¹ associated with –CH2 and CH3 groups. Our FT-IR findings is consistent with previous
studies (Somturk et al., 2016, Ildiz et al., 2017, Altinkaynak et al., 2017) and it was shown that Cu-hNs synthesis based on curcumin was synthesized in PBS medium. The elemental composition of Cu-hNs was detected by using EDX analysis. The peaks obtained by the EDX analysis confirmed the presence of Cu in the structure of hNF (Fig. 5). The characterization data confirm that curcumin based Cu-hNs have between 19 and 36 µm diameter and synthesized in PBS buffer.

3.2. Catalytic activity of Cu-hNs

In order to evaluate the catalytic activity of Cu-hNs, guaiacol was used as the model substrate. The study was conducted in the presence of H2O2 in the medium. Oxidation of guaiacol catalyzed by Cu-hNs was recorded at 470 nm by spectrophotometer (Fig. 6). According to Ildiz et al. (2017), and, Baldemir et al. (2017) the peroxidase-like activity of Cu-hNs depended on a Fenton-like reaction. This reaction is mainly based on the formation of Cu+1 ions as a result of the reaction of Cu2+ in the Cu-hNs with H2O2 and subsequent oxidation of guaiacol by the radical hydroxyl groups formed by the reaction of Cu+1 (Wu et al., 2016). Our study is agreement with previous studies.

3.3. Antimicrobial activity of Cu-hNs

The antimicrobial activity was determined against fish pathogen bacterial strains and the MICs were given in Table 1. According to our results, the Cu-hNs have effective antibacterial activity against Lactococcus garvieae, Aeromonas hydrophila, Vibrio parahaemolyticus. Also, the antimicrobial effect mechanism of Cu-hNs can be explained by the Fenton-like reaction. The free radicals formed as a result of the reaction described above cause oxidative damage to the bacterial membrane (Baldemir et al., 2017). Similar formed as a result of the reaction described above cause oxidative damage to the bacterial membrane (Baldemir et al., 2017). Similar

Table 1

| Isolates              | MIC (µg/mL) |
|----------------------|-------------|
| Aeromonas hydrophila | 30          |
| Vibrio parahaemolyticus | 30        |
| Lactococcus garvieae  | 30          |

4. Conclusion

In this study, we synthesized the curcumin based flower-shaped Cu-hNs and characterized. Cheap and one-step synthesized Cu-hNs have been exhibited effective antimicrobial activity against fish pathogen bacteria (Gram-positive and Gram-negative). Also the Cu-hNs have shown peroxidase-like catalytic activity against model substrate guaiacol by Fenton-like activity. We suggest that for Cu-hNs synthesis and its biomedical applications, curcumin can use instead of expensive molecules such as DNA and amino acids. In addition, studies on the synthesis and biomedical applications of Cu-hNs by using other plant extracts are recommended.

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