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Preparation and Antitumor-promoting Activity of Curcumin Encapsulated by 1,3-β-Glucan Isolated from Vietnam Medicinal Mushroom *Hericium erinaceum*

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The clinical application of curcumin in cancer treatment is considerably limited due to its serious poor delivery characteristics. In order to increase the hydrophilicity and drug delivery capability, we encapsulated curcumin into 1,3-β-glucan isolated from Vietnam medicinal mushroom *Hericium erinaceum*. The 1,3-β-glucan-encapsulated curcumin nanoparticles (Cur–Glu) were found to be spherical with an average size of 50 nm, being suitable for drug delivery applications. They were much more soluble in water not only than free curcumin but also than other biodegradable polymer-encapsulated curcumin nanoparticles. An antitumor-promoting assay was carried out, showing the positive effects of Cur–Glu on tumor promotion of Hep-G2 cell line in vitro.

Development of therapeutic modalities with no or minimal side effects to normal organs in cancer treatment is of great concern, not only among the scientific community, especially pharmacologists, biologists, and chemists, but increasingly among the general population. In this regard, a variety of natural dietary compounds were investigated. As a potential candidate, curcumin, a yellow compound isolated from rhizomes of the herb *curcuma longa*, has received considerable attention because of its putative cancer prevention and anticancer activities which are mediated through influencing multiple signaling pathways.[1–3]

Although curcumin proves to be remarkably nontoxic and has promising anticancer activities, the application in anticancer therapies is limited due to its serious poor delivery characteristics.[4,5] To deal with this obstacle, a variety of methods including the incorporation of curcumin into liposomes and into phospholipid vesicles are being studied.[6–8] More recently, the approach of biodegradable polymer nanoparticles has been developed.[9–11] This offers promising enhanced therapeutic performance of anticancer drugs by increasing their bioavailability, solubility, and retention time. These drug formulations are superior to traditional medicines with respect to controlled release, targeted delivery, and therapeutic impact.

Additionally, *Hericium erinaceum*, a traditional edible mushroom, has received considerable attention because of their biological activities.[12,13] *Hericium erinaceum* was also reported to have cytotoxic effects on cancer cell lines thanks to its polysaccharide 1,3-β-glucan.[14] With this in mind, we intended to encapsulate curcumin by polysaccharide 1,3-β-glucan isolated from *Hericium erinaceum*, for the purpose of increasing the solubility and anticancer activity.

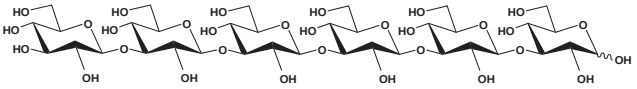


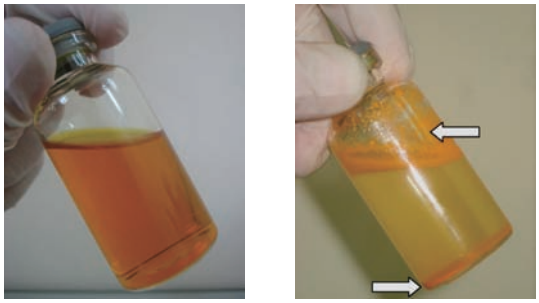
Figure 1. Structure of 1,3-β-glucan.

First, polysaccharides were isolated from the mushroom *Hericium erinaceum*. α-Amylase enzyme was used to break down the long chain of polysaccharides and eliminate 1,4-α-glucan. The resulting component was 1,3-β-glucan (Figure 1) (or curdlan) with short chain and molecular weight of 990, which was strongly supported by MS data (see Supporting Information 1[19]). The mass region of ion peaks was observed with peak-to-peak mass difference of 162 Da, consistent with the repeating unit of the 1,3-β-glucan.[15,16] Actually, the molecular peak was observed at 989 because the MS spectrum was taken in the negative ion mode (one proton lost).

Nanoprecipitation technique was used to prepare the 1,3-β-glucan-encapsulated curcumin nanoparticles. A 100-mg portion of 1,3-β-glucan was dissolved in 10 mL of double distilled water. To this solution, 10 mg of curcumin dissolved in 500 µL of absolute ethanol was added and stirred at room temperature for 12 h. This dispersion of nanoparticles was vacuum evaporated to eliminate the organic solvent completely. Larger aggregates and free 1,3-β-glucan polymers were removed by centrifugation at 5000 rpm for 15 min. The supernatant containing Cur–Glu nanoparticles was recovered by ultracentrifugation at 30000 rpm and then was subsequently lyophilized using a Labconco Freeze Dry System.

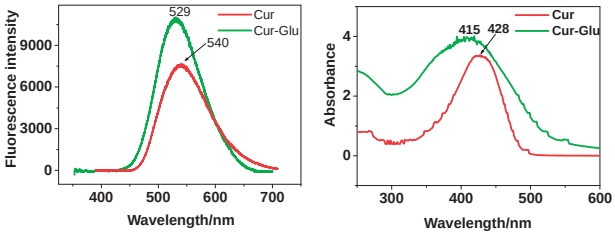
To estimate the amount of curcumin in 1,3-β-glucan micelles, curcumin was extracted with acetone. The concentration of curcumin was calculated using the calibration curve of acetone solution of curcumin. The absolute concentration of curcumin in filtered 1,3-β-glucan solution was found to be 4 mg mL^{-1}. For free curcumin, it immediately precipitates in aqueous medium due to very low solubility (ca. 20 µg mL^{-1}).[10] The Cur–Glu showed enormous improvements in aqueous solubility. The Cur–Glu also dissolves better than some other curcumin-loaded nanoparticles; 220-fold compared with the solubility of curcumin encapsulated by hydrophobically modified starch (HMS);[17] 2.5-fold compared with that of curcumin encapsulated by poly(lactic-*co*-glycolide) (PLGA).[10] The higher solubility may be a result of better compatibility between 1,3-β-glucan and curcumin.

Lyophilized Cur–Glu powder was reconstituted with water. It was seen that this powder dissolved to clear solution very quickly and easily, with no noticeable curcumin precipitates



(a) (b)

Figure 2. Solubility of (a) Cur–Glu and (b) Cur in water (arrows targeted at insoluble curcumin).



(a) (b)

Figure 3. (a) Fluorescence spectra and (b) absorption spectra of Cur and Cur–Glu.

(Figure 2a). This suggested that curcumin was indeed trapped in the 1,3-β-glucan micelles and that the complex of 1,3-β-glucan and curcumin could resist freeze-drying. Figure 2a shows the higher solubility of Cur–Glu in comparison with lower solubility of curcumin (Figure 2b).

In order to further confirm the formation of 1,3-β-glucan-encapsulated curcumin nanoparticles, the fluorescence and UV–vis spectra of curcumin and Cur–Glu solutions were recorded. As shown in Figure 3a, the emission peak of curcumin in water is at 540 nm. It is shifted to 529 nm upon the encapsulation by 1,3-β-glucan. Similarly, curcumin in ethanolic solution exhibits an absorption peak at 428 nm, while an aqueous solution of Cur–Glu shows a peak at 415 nm (Figure 3b). The blue shifts in the fluorescence and UV–vis spectra are likely due to the formation of intermolecular hydrogen bonding between curcumin and 1,3-β-glucan. The hydrogen bonding was also evidenced by the IR data of lyophilized Cur–Glu powder and 1,3-β-glucan powder (spectra omitted for brevity). Compared with that of pure 1,3-β-glucan, the IR spectrum of Cur–Glu shows a band shift from 3400 to 3417 cm^{-1}, which is probably due to the hydrogen bonding between –OH groups in curcumin and 1,3-β-glucan.

Figure 4 displays the FESEM (field-emission scanning electron microscope) image of 1,3-β-glucan-encapsulated curcumin nanoparticles, indicating spherical particles with an average size of 50 nm. There is a significant decrease in size of Cur–Glu compared with that of curcumin. This is probably because the short chain (only six D-glucose units) of 1,3-β-glucan was used to encapsulate curcumin. For delivering the drug via nanoparticles, their size should be tuned in such a way that the nanoparticles should be large enough to prevent rapid leakage into blood capillaries but escape the capture by

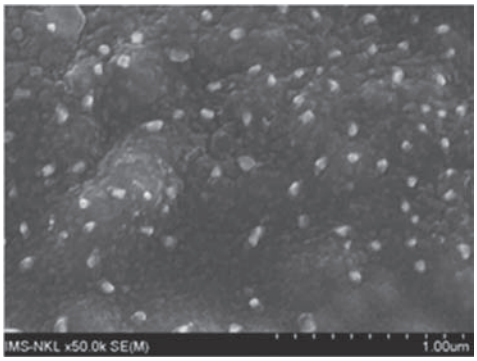


Figure 4. FESEM image of Cur–Glu particles.

macrophages lodged in the reticuloendothelial system.[18] 1,3-β-Glucan-encapsulated curcumin nanoparticles are in the optimal size range (below 200 nm), suitable for drug delivery applications.

Cell survival cytotoxicity experiments using sulforhodamine B were performed in order to determine the maximal doses of test materials for antitumor-promoting activity assays. Soft agar colony assay antitumor-promoting activity was estimated based on the inhibition of soft agar colony induction in the Hep-G2 cell line. The cells were cultured in 10% FBS-MEM (FBS: fetal bovine serum, MEM: minimum essential media) medium at 36.5 °C in an incubator with 5% CO_2 and 95% air. Cells growing logarithmically in a monolayer culture were trypsinized and suspended in 0.33% agar medium containing 10% FBS with or without samples at the concentrations of 25 $\mu g\,mL^{-1}$.

For antitumor-promoting assay, in duplicate six-well plates, 500 μL of the suspension (1×10^4 cells) was poured onto an agar layer containing the same concentration of sample (10 $\mu g\,mL^{-1}$) in 5% DMSO. Soft agar colonies of cells were investigated after 2 weeks' incubation under inverted microscope with a camera to compare the visual cell in their tumor formation, the tumor size and morphology. The inhibitory activities were the average of two independent experiments and expressed as a percentage of that of the control.

The results showed that there were no distinct differences of cell survival in cytotoxicity assay and the ratio of tumor promotion in antitumor-promoting assay with the glucan, curcumin alone was comparable to the control. However, there were clear changes in size and morphology of tumor between the control and all the samples tested, especially curcumin encapsulated with glucan. In the control wells, the tumor size was much larger and their surface was very rough in comparison to the tumor on the wells tested (see Supporting Information 2[19]). It was obvious that encapsulated curcumin had positive effects on tumor promotion of Hep-G2 cell line in vitro.

In conclusion, 1,3-β-glucan-encapsulated curcumin nanoparticles could be successfully prepared by nanoprecipitation. These particles have a good solubility in water, better than other biodegradable polymer-encapsulated curcumin nanoparticles reported in the literature. As spherical particles with the average size of 50 nm, they are also believed to be suitable for drug delivery applications. The antitumor-promoting assay shows the positive effects of Cur–Glu on tumor promotion of Hep-G2 cell line in vitro.

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19 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

